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Occupational Rhinitis

Diagnosis and Health-related Quality of Life

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University of Helsinki

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ACADEMIC DISSERTATION

To be publicly discussed, with the permission of the Medical Faculty of the University of Helsinki, in Auditorium 1 of the Haartman Institute,

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Content

List of original publications

This thesis is based on the following publications:

I Airaksinen L, Tuomi T, Vanhanen M, Voutilainen R, Toskala E. Use of nasal provocation test in the diagnostics of occupational rhinitis. Rhinology 2007, 45:40-6.

II Airaksinen L, Tuomi T, Tuppurainen M, Lauerma A, Toskala E. Inhalation challenge test in the diagnosis of occupational rhinitis. Am J Rhinol. 2008, 22:38-46.

III Airaksinen L, Luukkonen R, Lindström I, Lauerma A, Toskala E. Long-term exposure and health-related quality of life in patients with occupational rhinitis. J Occup Environ Med. 2009, 51:1288-97.

IV Lehto M, Airaksinen L, Puustinen A, Tillander S, Hannula S, Nyman T Toskala E, Alenius H, Lauerma A. Thaumatin-like protein (TLP) and baker's respiratory allergy. Ann Allergy Asthma Immunol. In press.

The publications are referred to in the text by their roman numerals.

Abbreviations

AR	allergic rhinitis
ASA	acetylic salicylic acid
E.g.	exempli gratia
EAACI	European Academy of Allergy and Clinical Immunology
EIA	enzyme immunoassay
FIOH	Finnish Institute of Occupational Health
HMW	high molecular weight
HPLC	high-performance liquid chromatography
HRQoL	health-related quality of life
ICT	inhalation challenge test
IUIS	International Union of Immunological Societies
LMW	low molecular weight
LTP	lipid transfer protein
MALDI	matrix-assisted laser desorption/ionization
MS	mass spectrometry
MW	molecular weight
OA	occupational asthma
OAR	occupational allergic rhinitis
OEL	occupational exposure limit
OR	occupational rhinitis
ORL	Otorhinolaryngology
PAR	perennial allergic rhinitis
PBS	phosphate buffered saline
RAND-36	RAND-36 item health survey 1.0
RAST	radioallergosorbent test
RUDS	reactive upper airways dysfunction syndrome
RQLQ	rhinitis quality of life questionnaire
SAR	seasonal allergic rhinitis
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SPT	skin prick test
TOF	time-of -flight
TLP	thaumatin-like protein
NPT	nasal provocation test
WAO	World Allergy Organization
1D	1-dimensional
2D	2-dimensional

Abstract

Aims

The aim of this thesis was to evaluate the use of challenge tests in occupational rhinitis (OR) diagnostics, to study the long-term health-related quality of life among allergic OR patients, and to study the allergens of wheat grain among occupational respiratory allergy patients. OR is a rhinitis mainly caused by work environment and not to stimuli encountered outside the workplace. It is different from work-exacerbated rhinitis, which is pre-existing or concurrent (allergic or non-allergic) rhinitis that are worsened by, but not mainly caused by, workplace exposures. OR can develop in response to allergens, inhaled irritants, or corrosive gases.

Methods

The thesis work consists of two retrospective analyses of patient files examined by either nasal provocation test (NPT, 165 patients) or by an inhalation challenge test (ICT, 829 patients). The causative agents and their provocation test results were retrospectively reviewed and nasal provocation test scoring positivity used and nasal secretion amount ≥ 0.2 g were compared in the results.

A cross-sectional questionnaire study was sent to 212 consecutive patients with allergic OR and to 414 controls. The study evaluated health-related quality of life (Rhinasthma and RAND-36 questionnaires), continuing allergen exposure, employment and possible re-education due to OR on average 10 years after the diagnosis.

Salt-soluble wheat proteins were purified using chromatographic methods and separated with gel electrophoresis. Serum from patients with baker's rhinitis, asthma, or both was immunoblotted with these proteins and the immunodetected proteins were characterized by tandem mass spectrometry. Skin prick tests (SPTs) were used to find out the relevance of the allergens among patients with baker's rhinitis or asthma to wheat.

Results

The diagnosed OR was mainly allergic rhinitis and the main causative occupational agents were flours and animal allergens. The non-IgE-mediated rhinitis reactions were less frequent in our challenge tests. Forty-seven percent of the patients tested with NPT (77/164) were diagnosed as having occupational rhinitis. The NPT was positive in 39% of the provocations done with occupational agents (125/318), whereas in 10% (19/193) placebo tests gave a positive nasal provocation reaction. Out of the positive scored nasal

challenge tests 111/117 had ≥ 0.2 g secretion amount. 302/303 nasal provocation tests the secretion had been < 0.2 g and the provocation test result was regarded as negative. In OR cases there was a clear difference between the occupational and placebo provocation tests. In the ICTs evaluated, rhinitis appeared in 13% (111/829) of challenged patients, while asthmatic reactions appeared in 25% of these patients. The placebo tests gave 2% positive rhinitis reactions in the ICTs. The tested agents were only partially similar in the ICT and NPT.

Continuing allergen exposure was related to decreased HRQoL on average 10 years after the diagnosis among patients with allergic OR, despite the use of rhinitis medications. The HRQoL of the patients, who were not occupationally exposed any longer, had a HRQoL mainly comparable to the healthy controls. The long-term employment rate was similar among the Finnish OR patients and the controls, but 17% of the OR patients had had re-education due to their OR.

Several wheat allergens were identified and purified and their clinical relevance was tested with skin prick tests. They included two new allergens that were found as clinically relevant with baker's rhinitis and asthma.

Conclusions

Both nasal challenges and inhalation challenges were found to be safe tests, as no serious reactions or anaphylactic reactions have appeared in the tests in our institute. The inhalation challenge tests have considerably resource-intensive methodology. However, if both upper and lower airway studies are needed, the evaluation of nasal symptoms and signs together with bronchial reactions saves time and expense compared with the organization of multiple individual challenges. The scoring criteria used matched well with the weighted amount of discharge ≥ 0.2 g and in most cases gave comparable results. The challenge tests are valuable tools when there is uncertainty whether the patient's exposure should be discontinued or he or she should change job. However in non-IgE related rhinitis the specific mechanisms are often unknown and mostly the non-IgE-mediated agents cause more often asthma than rhinitis. The rhinitis reactions may be milder than in IgE-mediated rhinitis and therefore they may be more difficult to recognize. There is still need to find out the most relevant methods for non-IgE mediated rhinitis.

We found that continuing exposure decreases HRQoL among patients with allergic OR despite of rhinitis medications still approximately ten years after the diagnosis. HRQoL among OR patients without any longer occupational exposure were mainly similar than that of the healthy controls. This highlights the importance of the reduction and cessation of occupational exposure. To achieve this, re-education in many cases (17%) has been necessary.

The knowledge of the new clinically relevant proteins can be used in the future in the development of better diagnostic preparations and perhaps treatment for baker's rhinitis and asthma.

Tiivistelmä (Summary in Finnish)

Tavoitteet

Väitöskirjatyön tavoitteena oli arvioida ammattinuhan altistuskoemenetelmiä, selvittää allergiseen ammattinuhaan sairastuneiden pitkäaikaista elämänlaatua sekä kartoittaa ammattinuhan yhden pääaiheuttajan, vehnän, allergeeneja vehnäallergista jauhonuhaa tai -astmaa sairastavilta potilailta. Ammattinuhalla tarkoitetaan työssä esiintyvän tekijän pääasiallisesti aiheuttamaa nuhaa. Se tulee erottaa työn pahentamasta, (tai samanaikaisesta muusta) nuhasta, jossa työympäristö siis ei ole pääasiallinen nuhan syy.

Päämenetelmät

Väitöskirjatyössä tehtiin kaksi takautuvaa tutkimusta ammattinuhaan sairastuneiden potilaiden potilastiedoista, joita oli diagnosoitu työperäisin altistustestein (nenäaltistustestein 165 ja kammioaltistustestein 829 potilasta). Ammattinuhan aiheuttajat ja altistuskoetestitulokset arvioitiin takautuvasti ja käytettyä nenäaltistuskokeiden (pisteytys) positiivisuutta verrattiin ≥ 0.2 g nenän eritemäärän kriteeriin.

Keskimäärin kymmenen vuotta aiemmin diagnosoiduille 212 allergista ammattinuhaa sairastavalle potilaalle, sekä 414 iän ja sukupuolen suhteen samankaltaiselle satunnaistetulle verrokkihenkilölle tehtiin postitse kyselytutkimus. Kysely selvitti terveyteen liittyvää elämänlaatua (RAND-36 ja Rhinasthma), potilaiden altistumisen jatkumista, työssäkäyntiä ja mahdollista uudelleenkoulutusta.

Vehnän vesiliukoisia valkuaisaineita puhdistettiin ja eroteltiin (geelifiltraatio, kromatografiat) sekä tunnistettiin leipurin nuhan ja astman allergeeneiksi potilasseerumien avulla (SDS-PAGE, massaspektrometria). Tunnistettujen proteiinien merkitystä allergeeneina tutkittiin ihopistotestien avulla 20 työssään vehnälle allergisilla nuha/astmapotilaalla ja 10 terveellä verrokkihenkilöllä.

Saavutetut tulokset

Todetut ammattinuhat olivat yleensä allergista nuhaa, ja tavallisimmat ammattinuhan aiheuttajia olivat jauhot ja eläimet. Ei-allergista nuhaa tuli testeissä esiin selvästi harvemmin kuin IgE-välitteistä allergiaa. Ammattinuha todettiin 47% (77/164) nenäaltistustestein tutkituista potilaista. Työperäisillä aineilla tehdyistä nenäaltistuskokeista 39% (125/318) oli testipositiivisia, kun taas lumeaineella tehdyistä nenäaltistuksista 10% (19/189) oli positiivisia. Ammattinuhatapauksissa todettiin selvä ero

työperäisen ja lumealtistuksen tuloksen välillä. Positiiviseksi luokitelluissa nenäaltistuskokeissa oli 111/117 ≥ 0.2 g eritysmäärä. Negatiiviseksi luokitelluissa altistuskokeissa eritemäärä oli < 0.2 g 302/303 testissä. Kammioaltistustesteissä 13% (111/829) potilaalle tuli positiivisen testin kriteerit täyttävä nuhaoireisto, kun taas astmareaktiot olivat yleisempiä (25%). Kammioaltistuksien lumetesteistä 2% aiheutti nuhareaktion. Nenä- ja kammioaltistuksissa testatut aineet olivat vain osittain samoja.

Jos työperäinen altistuminen allergeeneille jatkui noin 10 vuotta ammattinuhan diagnosoinnin jälkeen, terveyteen liittyvä elämänlaatu oli ammattinuhaan sairastuneilla alentunut, nuhalääkityksestä huolimatta. Ei-altistuvien potilaiden elämänlaatu oli pääosin samankaltainen kuin terveillä ei-allergisilla verrokkihenkilöillä. Ammattinuhapotilaat ja verrokkihenkilöt eivät eronneet toisistaan työssäkäynnin suhteen, mutta merkittävä osa (17 %) ammattinuhapotilaista oli ammattitaudin takia uudelleenkoulutettuja.

Useita vesiliukoisia allergeeneja puhdistettiin vehnästä ja tunnistettiin. Niiden merkitystä hengitystieallergeeneina tutkittiin leipurin nuhaa ja / astmaa sairastavilla vehnäallergisilla potilailla ihopistotestein. Tutkimuksessa löydettiin kaksi uutta merkityksellistä vehnän allergeenia leipurin ammattinuhassa ja/ tai astmassa.

Johtopäätökset

Työperäisillä aineilla tehtävät nenä- ja kammioaltistuskokeet todettiin analyyseissä turvallisiksi testeiksi, koska vakavia, vaikeasti hoidettavia allergiareaktioita ei niissä ollut esiintynyt Työterveyslaitoksen historiassa. Altistustestit ovat arvokkaita, kun on epävarmaa voiko altistuminen jatkua tai tuleeko potilaan vaihtaa työtä. Työterveyslaitoksen käyttämä pisteytyskriteeri antoi pääasiassa yhteneväisen tuloksen eritemittauksen kanssa, joka tukee oletusta eri testimenetelmien pääosin yhteneväisistä tuloksista työperäisen allergian testauksissa. Vaikka kammioaltistuskoe vaatii paljon resursseja, voidaan kammioaltistuksessa yhdistää keuhkoputkialtistus ja nenäaltistus, mikä säästää aikaa ja kustannuksia verrattuna kahteen eri tutkimukseen. Ammattinuhat ovat pääosin allergista nuhaa. Ei-allergisten ammattinuhan aiheuttajien osalta nuhan mekanismit ovat pääosin tuntemattomia, ja näiden pääasiassa ns. pienimolekyylisten aineiden suhteen astmareaktiot ovat tavallisempia löydöksiä kuin nuhat altistuskokeissa. Nuhareaktiot voivat olla myös lievempiä kuin allergisessa nuhassa, jonka takia niitä voi olla vaikeampi tunnistaa. Ei-allergisen nuhaan sopivimpien tutkimusmetodien löytämiseen on edelleen tarvetta.

Koska altistumisen jatkumisen todettiin olevan yhteydessä terveyteen liittyvään elämänlaadun alentumiseen vuosienkin kuluttua diagnoosista nuhalääkityksestä huolimatta ja altistumisen loputtua terveyteen liittyvä elämänlaatu oli pääosin samanlainen kuin terveillä verrokkihenkilöillä, väitöstutkimus korostaa altistumisen vähentämisen tai lopetuksen tarvetta ammattinuhapotilailla. Altistumisen vähentämisen mahdollistamiseksi uudelleenkoulutus oli ollut ammattinuhaan sairastuneilla melko usein tarpeen.

Tietoa uusista vehnän työperäistä hengitystieallergiaa aiheuttavia merkittävistä valkuaisaineista voidaan jatkossa hyödyntää kehitettäessä leipurin nuhaa ja astmaa testaavia testejä ja kenties hoitoja.

1 Introduction

"When you come to a patient's house, you should ask him what sort of pains he has, what caused them, how many days he has been ill, whether the bowels are working and what sort of food he eats." So says Hippocrates in his work *Affections*. I may venture to add one more question: what occupation does he follow? says Bernardino Ramazzini 1700 in *Diseases of Workers*, *Preface* (Salvaggio, 1990; Erensen, 2009).

Today occupational rhinitis (OR) is linked to the fields of occupational medicine, otolaryngology, allergology and immunology, toxicology and industrial hygiene. The compensation and measures of rehabilitation are dependent on the insurance companies' acceptance. In addition both the OR diagnostics as well as the decisions of the insurance companies are guided by the legislation.

This thesis work was started from the need to update the knowledge of OR diagnostics results with challenge tests, causative allergens and agents and the sensitivity of the tests in order to improve the efficacy of the Otorhinolaryngology (ORL) work field in our institute. All the challenge test agents used during the study and the test results both in nasal and inhalation challenge tests had not been assessed recently, and the inhalation challenge test had not been previously evaluated at all to our knowledge. In addition, there is a long-lasting need for a comparison of the various criteria of challenge tests, as there are various nasal challenge test methods which are not harmonized. The study describes and characterizes diagnostics of OR cases in Finnish institute of Occupational Health, and the role of provocation tests in it.

Occupational diseases have a fundamental role for the patients, as these diseases can be a direct threat to ones decreased income, unlike with most diseases. As physicians examining these patients, we wanted to find the impact on the OR to the patients' long-term health-related quality of life and livelihood, as these aspects had not been previously evaluated and as major changes in work like relocation or re-education are often needed because of the occupational diseases.

2 Review of the literature

2.1 Definitions of rhinitis

Rhinitis is defined as an inflammation of the lining of the nose, and it is characterized by nasal symptoms including anterior or posterior rhinorrhea, sneezing, nasal blockage and/or itching of the nose (International Rhinitis Management Working Group, 1994). By definition the symptoms occur during two or more consecutive days for more than 1 h on most days. Because there is no agreed international standard method to objectively diagnose nasal inflammation, the symptom evaluation has been assigned a great importance in the diagnosis of OR (Hellgren *et al.*, 2003).

2.2 Definition of allergic and non-allergic rhinitis

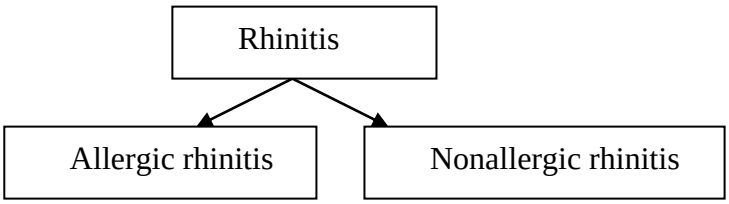
Allergy is defined as a hypersensitivity reaction initiated by immunologic mechanisms. An allergy can be antibody-mediated or cell-mediated. In most patients, the antibody typically responsible for an allergic reaction belongs to the IgE isotype, and these patients may be said to suffer from IgE-mediated allergy. Inhalation of large amounts of protein, as in mold, grain dust, etc., stimulates the immune system to produce antibodies mainly of the IgG, IgA, and IgM isotype. There is a relation between degree of exposure and antibody concentration (Johansson *et al.*, 2004).

The European Academy of Allergy and Clinical Immunology (EAACI) position statement proposes that non-IgE-mediated allergic reactions be subdivided into those in which the reaction is initiated predominantly by mechanisms associated with allergen-specific antibodies other than IgE, and those in which a cellular response is predominant (Johansson et al., 2001). The World Allergy Organization (WAO) revised nomenclature for allergy states that when other mechanisms than IgE can be proven behind the hypersensitivity, like as in hypersensitivity to aspirin, the term nonallergic hypersensitivity should be used (Johansson *et al.*, 2004).

Hypersensitivity, in the WAO recommendations, is suggested to be used to describe objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons (Johansson *et al.*, 2004).

Allergic rhinitis (AR) is clinically defined in the World Health Organization Allergic Rhinitis and its Impact on Asthma (ARIA) workshop update as a symptomatic disorder of the nose induced after allergen exposure by an IgE-mediated reaction (Bousquet *et al.*, 2008). Other than IgE-mediated rhinitis reactions are defined as non-allergic rhinitis (Figure 1).

Figure 1. *Allergic rhinitis*

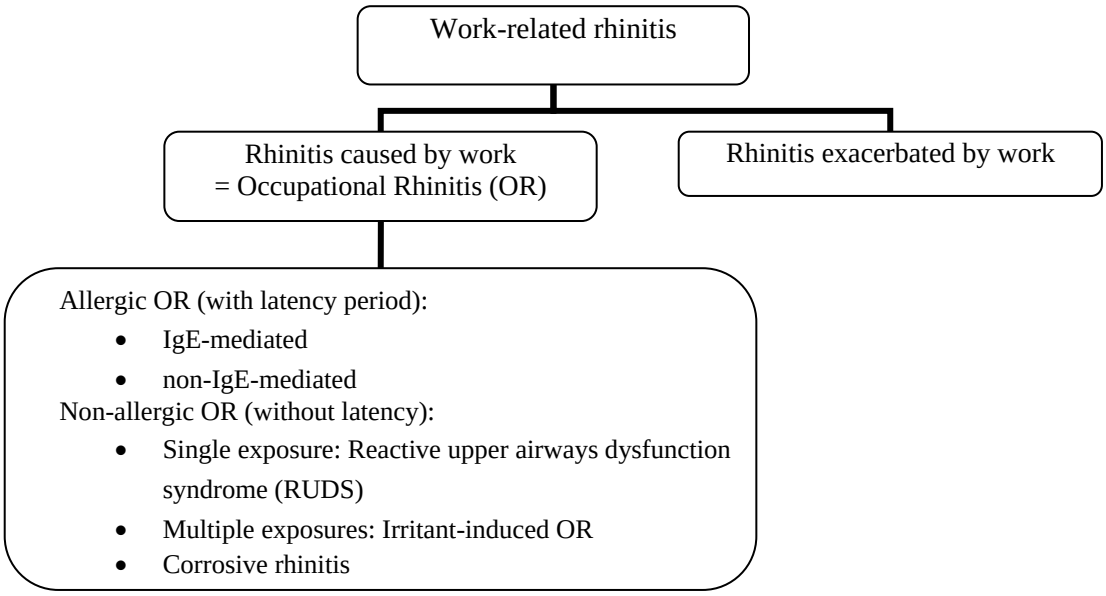


The diagnostic criteria are different for epidemiological studies, and the criteria used affect the reported prevalence. Several standardised questionnaires exist and each of them has different sensitivity and specificity (Charpin *et al.* 1996). The specificity and sensitivity of AR questions for the epidemiologic studies in Finnish has been studied from the Tuohilampi questionnaire (Susitaival and Husman, 1996). A question of "were you told by a doctor that you have hay fever or nasal allergy" has a specificity of 0.93 and sensitivity of 0.52, as compared to symptoms of AR during the preceding year at interviews by the chest physician and at least one wheal reaction with a mean diameter of at least 3 mm in SPT and/or specific IgE test (Phadiatop combi®) greater than 0.35 kU/l (Kilpeläinen *et al.*, 2001).

2.3 Definitions of occupational rhinitis

The classification of occupational rhinitis (OR) has been recently revised after an initiative to evaluate the definition and the classification in the scientific literature (Castano and Theriault, 2006). An operational definition and classification of OR as well as a diagnostic algorithm for OR has been proposed in 2008 by the EAACI Task Force on Occupational Rhinitis (Moscato *et al.*, 2008). The work has been published as an EAACI position paper (Moscato *et al.*, 2009, Figure 2). They define: OR is an inflammatory disease of the nose, which is characterized by intermittent or persistent symptoms (i.e., nasal congestion, sneezing, rhinorrhea, itching), and/or variable nasal airflow limitation and/or hypersecretion arising out of causes and conditions attributable to a particular work environment and not to stimuli encountered outside the workplace.

***Figure 2**. EAACI consensus definition of OR (Moscato et al., 2009)*



Allergic occupational rhinitis

Allergic occupational rhinitis has been further divided in the following way:

1. IgE-mediated OR: can be caused by a wide variety of high molecular weight (HMW) agents (i.e. glycoproteins from vegetal and animal origin) and some low molecular weight agents (LMW) for which an IgE-mediated mechanism has been proven, such as platinum salts, reactive dyes, and acid anhydrides.

2. Non-IgE-mediated OR: can be induced by LMW agents (e.g. isocyanates, persulphate salts, wood dusts) acting as haptens for which the allergic mechanism has not yet been fully characterized (Moscato *et al.*, 2008).

Non-allergic occupational rhinitis

Non-allergic OR has been stated to encompass rhinitis caused by work environment through irritant, non-immunological mechanisms. Single or multiple exposures high concentrations of irritant compounds like ozone or chlorine can lead to transient or persistent symptoms of rhinitis (Leroyer *et al.*, 1999; Hoffman *et al.*, 2004). As the situation is much like reactive airways dysfunction syndrome (RADS), the term reactive upper airways dysfunction syndrome (RUDS) has been proposed (Meggs, 1994; 1997).

The term irritant-induced OR may also refer to symptoms of rhinitis reported by subjects repeatedly exposed to irritants, without identifiable high concentrations of them (Moscato *et al.*, 2009).

Patients with nasal septal perforations of occupational origin exhibit the clinical and histopathological features of rhinitis whereby they should be categorized as rhinitics. It has been suggested lately, that this rhinitis should be considered as a type of irritant-induced OR and classified as corrosive rhinitis (Castano *et al.*, 2007).

Other definitions

An OR definition, introduced earlier to the EAACI consensus paper has been, for example, the episodic work-related occurrence of sneezing, nasal discharge, and nasal obstruction (Bardana, 1995). The mechanisms causing OR have been classified in some expert opinion to heightened olfactory awareness, nonspecific inflammation of the nose, exposure to a high concentration of irritating and soluble chemical gases and IgE mechanisms (Slavin, 2003).

The classification to IgE-mediated allergy and other forms of non-IgE-mediated immunologically mediated diseases has been somewhat inconsistent in the literature. In the occupational setting, the EAACI position papers on occupational rhinitis (Moscato et al., 2008; Moscato et al., 2009) define occupational AR to include IgE- mediated rhinitis and non-IgE-mediated rhinitis forms, in which immunologically mediated mechanisms are suspected. On the other hand, in another definition, AR is clinically defined as a symptomatic disorder of the nose induced after allergen exposure only by an IgE-mediated reaction (Bousquet et al., 2008), and those reactions other than IgE-mediated are defined as non-allergic rhinitis. WAO revised the nomenclature for allergy states so that when other mechanisms than IgE can be proven behind the hypersensitivity, like as in hypersensitivity to aspirin, the term nonallergic hypersensitivity should be used (Johansson et al., 2004).

The Finnish OR statistics consider both IgE and non-IgE-mediated forms as AR and allergic respiratory diseases in the yearly reports (Karjalainen *et al.*, 2001b). The non-allergic OR (without latency) cases diagnosed in Finland have been solitary cases only.

The Act and Ordinance on Occupational Diseases in Finland defines OR as a disease probably primarily and mainly caused by any physical factor, chemical substance or biological agent at work, encountered in the course of work done under contract of employment, in the public service or in public office or as an agricultural entrepreneur (Finnish Ministry of Social Affairs and Health, 1989a, 1989b).

2.4 Causes of occupational rhinitis

The etiological agents causing OR have been extensively reviewed (Siracusa *et al.*, 2000; van Kampen *et al.*, 2000), and the various occupations relative risk of OR in Finland during 1986-1991 have been analyzed from the Finnish Register of Occupational Diseases and Finnish censuses as well (Hytönen *et al.*, 1997).

Flours and foods as a cause of occupational rhinitis

The most common cause of OR in Finland is flour dust (Karjalainen *et al.*, 2008). The flour dust means dust from finely milled or otherwise processed cereal. Cereal allergens, that spread through the air and inhaled can cause occupational inhalation allergy. Flours are the main cause of baker's rhinitis (and baker's asthma), and specific IgE is most often found in baker's rhinitis against wheat, rye, barley or oats. These species are taxonomically closely related and there is strong cross-antigenity between them (Baldo *et al.*, 1980; Sandiford *et al.*, 1995). Wheat is one of the most commonly used flour in the western world and the main cause of baker's rhinitis and asthma in these countries (Brisman, 2002). Other reported flours that have been stated as causes of OR are maize (Borghesan and Borghesan, 2005), corn dust (Park and Nahm, 1997), lupine flour (Campbell *et al.*, 2007) soybean and other botanically relative legumes (De Zotti *et al.*, 1988; Martinez San Ireneo *et al.*, 2000). In addition, other baking ingredients, spices, enzymes, other additives, mold, insects, mites and other contaminations of foodstuffs can cause allergy or irritation rhinitis (Brisman, 2002).

Other food causing OR have been for example: clam and shrimp (Desjardins *et al.*, 1995), lobster (Lemiere *et al.*, 1996), milk proteins (Toskala *et al.*, 2004), egg proteins (Leser *et al.*, 2001), mushrooms (Symington *et al.*, 1981) and seeds (Keskinen *et al.*, 1991; Vandenplas *et al.*, 1998).

Occupational wheat allergens

There are nine characterized allergens in wheat seed (Tri a 14 non-specific lipid transfer protein 1, Tri a 18 agglutinin isoleucin 1, Tri a 19 omega-5 gliadin, Tri a 25 thioredoxin, Tri a 26 glutenin, Tri a 27 thiol reductase homologue, Tri a 28 dimeric alpha-amylase inhibitor 0.19, Tri a 29 tetrameric alpha-amylase inhibitor CM1/CM2, Tri a 30 tetrameric alpha-amylase/trypsin inhibitor CM3), accepted on the International Union of Immunological Societies (IUIS) Allergen Nomenclature sub-committee list of certified protein allergens. They are listed on the website www.allergen.org (Marsh *et al.*, 1986). In addition there other wheat allergens introduced (e.g. Weiss *et al.*, 1993; Garcia-Casado *et al.*, 1996; (Sanchez-Monge *et al.*, 1997) and some new recently (Constantin *et al.*, 2009, de Gregorio *et al.*, 2009) and most of them are included in other web-base allergen databases like www.allergome.org (Mari *et al.*, 2009).

Animal proteins as a cause of occupational rhinitis

Cattle are among the leading causes of occupational rhinitis (OR) (Heutelbeck *et al.*, 2007). In farm surroundings storage mites are also often relevant allergens (Terho *et al.*, 1987). Nowadays several laboratory animal derived dusts, especially from mice and rats are frequent causes of OR (Bush and Stave, 2003). Some other OR cases has been reported to roe deer (Carballada *et al.*, 2006) and to goat (Ferrer *et al.*, 2006). Various insects, mites, spiders, flies, cockroaches and worms are also well-recognized causes of OR.

Other organic causes of occupational rhinitis

Earlier, natural rubber latex, mainly in powdered gloves, has been an important allergen especially in health care. The development of less allergenic gloves has greatly decreased the problem. Various enzymes are important and potent causes of OR and their inhalative uptake should be avoided (Baur, 2005). Plant pollens, plant and seed dust may cause sensitization and OR in persons handling them at work (Martinez San Ireneo *et al.*, 2000; Groenewoud *et al.*, 2002). For example various beans and legumes have been reported to cause OR (Osterman *et al.*, 1982; Codina *et al.*, 2000; Daroca *et al.*, 2000; Martinez San Ireneo *et al.*, 2000). Wood-derived natural rubber latex, obeche and red cedar, and some other wood dusts are known as sensitizing causes of OR. Some molds can cause IgE-mediated occupational rhinitis (Merget *et al.*, 2008), but yet molds have been found only rarely causing sensitization, and that sensitization to them is likely associated to other allergies (Reijula *et al.*, 2003). In addition some hard wood dusts are causes of nasal irritation that may lead to occupational carcinogenesis (Alberty *et al.*, 2009).

Inorganic causes of occupational rhinitis

Among the first studies of occupational inorganic rhinitis were studies from 1941-1955 of paraphenylenediamine, a chemical used in hair and fur dying, that was found to be a cause of occupational rhinitis (Silberman and Sorrell, 1959). Some other chemicals causing occupational rhinitis (OR) are for example various isocyanate-derivatives (Baur *et al.*, 1984; Baur *et al.*, 2001), persulphate (Moscato *et al.*, 2005), thioglycolates (Leino *et al.*, 1998), formaldehyde, that can also exert nasal irritant reactions (Pazdrak *et al.*, 1993), glutaraldehyde (Palczynski *et al.*, 2001), newly found component tolyltriazole in metal-cutting fluids (Graff *et al.*, 2008), methacrylates and cyanoacrylates (Weytjens *et al.*, 1999; Torres *et al.*, 2005).

Organic acid anhydrides are potent sensitizers and potent causes of OR as well as asthma (Kimber and Dearman, 2002) like reactive dyes (Alanko *et al.*, 1978; Nilsson *et al.*, 1993), henna (Majoie and Bruynzeel, 1996), some metals (Cristaudo *et al.*, 2005; Malo, 2005), medicaments (Malet *et al.*, 1992; Moscato *et al.*, 1995) and disinfectant chloramine-T (Blasco *et al.*, 1992). Some case reports of occupational AR to tetrazene

(Burge *et al.*, 1984), ninhydrin (Piirila *et al.*, 1997) and benzisothiazolin-3-one have also been reported (Moscato *et al.*, 1997).

2.5 Diagnosis of occupational rhinitis

Legislation in Finland

The decree on Occupational Diseases in Finland defines that the diagnosis of occupational disease requires medical examination where there is a sufficient knowledge of the exposure at work and a medical doctor familiar with occupational diseases diagnostics in charge (Finnish Ministry of Social Affairs and Health, 1989b).

The decree lists for example the following chemical factors where rhinitis or irritation of mucous membranes is given as one of the typical forms of diseases:

- cobalt and its compounds as allergens
- chromium and its compounds as allergens or as local irritative/ corrosive cause of rhinitis or as causes of cancer of nasal sinuses
- nickel and its compounds as allergens causing rhinitis or as cause of cancer of the nasal sinuses
- chlorine, chromine bromine, fluorine as irritants and corrosive to mucous membranes
- cyano compounds: respiratory diseases caused by isocyanates
- sulfur dioxide and sulfur acid as irritatives and as causing inflammatory symptoms of mucous membranes
- nitrogen oxide, nitric acid and ammonia as acute irritants of respiratory tract
- inorganic bases and their anhydrides as causes of acute corrosion symptoms of mucous membranes
- halogenated derivates of hydrocarbons: irritative respiratory symptoms caused by freons
- rhinitis caused by formaldehyde
- respiratory allergies caused by antibiotics

The decree names the following agents as causes of rhinitis without definition of the causative mechanism:

- plastics and the synthetic resins and the substances and intermediates involved in their production
- organic acids and acid anhydrides
- reactive dyes
- moulds

The decree of occupational diseases states also, that occupational rhinitis can be caused by

- organic dusts, i.e. flours, grain, wood dusts and materials, animal dander and other exposures of animal origin, dusts of organic fibres, enzymes, natural resins like colophony, natural rubber latex.

The disease is considered to have begun during the time the patient has first sought medical help from a doctor because of the occupational disease symptoms.

Occupational exposure limits in Finland

The Finnish Ministry of Social Affairs and Health publishes maintains and updates the Finnish occupational exposure limit (OEL) value lists, which is a list of concentrations of impurities in workplace air, that are known to be hazardous (HTP values in Finnish) to the workforces safety, health or breeding health. They are confirmed by the law and Act of safety at work in Finland (Finnish Ministry of Social Affairs and Health, 2002) and the related publications are renewed frequently (Finnish Ministry of Social Affairs and Health, 2009). The effects that the exposure may cause to sensitive groups (e.g. atopics or various patient groups) are usually not taken into account while the limits have been set, so they need to be considered separately.

Irritation of skin, eyes and airways by the impurities of workplace air has been taken into account in the values of the OEL values. The Finnish OELs are values which the employer needs to pay attention to while assessing the worker's exposure to air impurities.

The harmful or hazardous effects of air impurities depend on their concentration and on exposure time. Therefore, the OELs are confirmed for the time-weighted averages for 8 hours, 15 minutes and/ or momentary exposures.

According to the OEL values 2009 (www.ketsu.net/htp/HTP2009.pdf), the following titles relevant to OR are to be updated in 2011:

- Flours, grain dusts: airways sensitization
- Wood dust: airways sensitization and airways irritation
- Endotoxines: irritation and other effects on airways
- Enzymes: airways sensitization
- Inhaled dust: effect on airways
- Pyromellitic dianhydride and tetrahydrophtalic anhydride: airways sensitization.

Medical history and exposure at work

The purpose of the medical history is to confirm the existence of rhinitis and to evaluate the link to work.

The medical and work history covers

1) The types and amounts of the exposures at work, their potential to sensitize or irritate the airway mucosa. In Finland, the airway sensitization knowledge of the agents used in the workplace and in the case of chemicals, their related safety data sheets are due to be available in the workplace (Finnish Ministry of Social Affairs and Health, 2001).

2) Latency of rhinitis after the beginning of the exposure

3) Individual medications and other diseases history

4) Individual symptoms of rhinitis (rhinorrhea, sniffing, nasal stuffiness, nasal itch), their intensity, time-scale and relation to work and various exposures

In addition nasal examination and related tests are needed to exclude bacterial sinusitis and other obvious non-occupational causes of rhinitis behind the symptoms.

Immunological tests in occupational rhinitis diagnostics

Skin prick test, Prick-prick test

The sensitization to occupational agents can be tested by means of skin prick test (SPT) or /and assessment of serum allergen-specific IgE antibodies. The different criteria for the positivity have been used in various studies. In Finland national guidelines have been made for the interpretation of the test results (Juntunen-Backman *et al.*, 1995). In this recommendation, the mean skin wheal diameter needs to be ≥ 3 mm and or larger than histamine wheal, that needs to be ≥ 3 mm. In an EAACI position paper, a SPT is considered to be positive when the mean wheal diameter is > 3 mm (area > 7 mm) (Mailing, 1993). A criterion that relates the allergen wheal to the size of a histamine wheal can compensate for the differences for prick observer differences and is appropriate for epidemiological studies especially for children (Meinert *et al.*, 1994). A positive immunological test may appear in a substantial proportion of exposed asymptomatic individuals. On the other hand, negative test results make the diagnosis of IgE-mediated OR unlikely, provided that appropriate allergens have been tested. The main limitation of immunological tests in the investigation of occupational allergy results from the lack of standardized, commercially available extracts, especially LMW agents (Moscato *et al.*, 2008). Prick-prick tests have been introduced due to the poor standardization of food extracts commonly available. In prick-prick test the lancet pricks the food item first and after that the same lancet thus containing food material is used for skin prick test. These tests are used in situations for which the common commercial test agents are not available, or are found to be poor, such as with the grains (Romano *et al.*, 1995; Sander *et al.*, 2004).

The SPT with individual flour extracts has been shown to be superior to commercially available SPT solutions to rye or wheat flours in the diagnosis of occupational inhalant allergies (Tasman *et al.*, 1999). A small study has shown that results of the prick-prick method give comparable results and are faster and less expensive compared to traditional SPT method (Zawodniak *et al.*, 2003).

Specific IgE detection: ImmunoCAP, ELISA, Immunospot

Specific IgE detection from the sera has been classically made by a radioallergosorbent test (RAST), but a superior test named ImmunoCAP® has replaced it with wider sensitivity and specificity (Leimgruber *et al.*, 1991). Dot blot (immunospot) has also been at times used in the detection of specific IgE-reactions. The Immunospot assay is based on a modified version of the Enzyme ImmunoAssay (EIA or ELISA) technique used to detect the presence of an allergen in a sample. In EIA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step, a substance is added so that the enzyme can convert to some detectable signal (Paulie *et al.*, 2006).

Rhinitis provocation tests

The main indications for using the nasal provocation test (NPT) are the assessment of allergic symptoms in both OR and immunotherapy. NPT has also been used for the diagnosis of nonallergic OR caused by irritating occupational substances (Malm *et al.*, 2000; Gosepath *et al.*, 2005), for a diagnosis of acetylic salicylic acid (ASA)-induced asthma (Milewski *et al.*, 1998), for rhinitis medication studies (Andersson *et al.*, 1991) as well as for research (Baroody *et al.*, 2008). As the diagnosis of OR has substantial financial and work-related consequences, the relationship with the work exposure needs to be ascertained with provocation tests (Moscato *et al.*, 2009).

Provocation test criteria

There are no uniformly accepted criteria for the evaluation of nasal provocation test reactions (Moscato *et al.*, 2009). In addition, the test has been done either unilaterally or bilaterally.

Various symptoms and findings have been traditionally used as the main criteria of the positivity of nasal provocation test reactions. For example, the sneeze count, inspected nasal blockage (congestion), itching or burning of the nose, palate or throat and lacrimation have been used (Milewski *et al.*, 1998) or symptom score of nasal itching, sneezing, and rhinoscopic nasal obstruction, rhinorrhea, and mucosal oedema (Moscato *et al.*, 2005). Various symptom scores from 1976-1994 are summarized in a Finnish thesis (Hytönen, 1997). In Finland, the FIOH has used the scoring criteria for nasal blockage and

rhinorrhea as the main criteria for the diagnosis of OR since the 1970's (Hytönen and Sala, 1996). Before that symptom score with unilateral itching, swelling of the mucosa, watery discharge or sneezing was used. It included criteria that test with physiological saline in another nostril and another provocation test with lactose or saline were considered as negative (Kanerva and Vaheri, 1993). EAACI position paper of provocation tests and of occupational rhinitis state that there is general agreement that both subjective and objective indices must be considered (Melillo *et al.*, 1997; Moscato *et al.*, 2009).

Various objective instruments have been introduced to value the nasal reactions. Acoustic rhinometry and anterior rhinomanometry have been the main tools in the hands of rhinologists. In acoustic rhinometry, a minimum cross-sectional area of the nose has been used often, as well as total nasal volume from 1 to 5 cm (Litvyakova and Baraniuk, 2001; Hilberg, 2002; Kim *et al.*, 2006). Recently, nasal volume from 2-5 cm depth has been recommended for the criteria evaluation for nasal mucosal reactions (Clement and Gordts, 2005). In addition, nasal peak inspiratory flow, as well as optical rhinometry and rhinostereometry have been introduced for the evaluation of nasal provocation results (Hallen and Graf, 1999; Wustenberg *et al.*, 2004).

Measurement of secretion has been introduced as one objective and relevant measurement of NPT, and in a unilateral test, it has shown to be slightly superior to acoustic rhinometry and rhinomanometry (Pirilä and Nuutinen, 1998).

In healthy normal controls, the baseline nasal reactivity is rather low (Baroody *et al.*, 1999), but the baseline characteristics vary in various study populations. In one study with ASA-intolerant asthmatics, baseline nasal reactivity with rhinomanometrically measured fluctuation of the nasal flow > 40% before the test or total nasal obstruction excluded 19.6 % of the study population (Milewski *et al.*, 1998). By contrast to bronchial hyperreactivity in asthma, nasal hyperreactivity is scantly documented in OR so far (Moscato *et al.*, 2009).

Challenge test methods

The nasal challenge test is the standard test to confirm the causative role of a specific agent and a rhinitis reaction. In nasal challenge tests, the exposure agent can be applied to either one or both nostrils by dropping, spraying, direct application, sniffing or with special devices. Inhalation challenge tests are seldom reported to be used in the diagnosis of OR, but it is a valuable method in the simultaneous evaluation of patients with both lower and upper airways symptoms (Moscato, *et al.* 2005, Castano *et al.*, 2009). In special cases workplace challenges are an option that might be considered when specific challenges are not possible, although the difficulty of standardization and therefore lack of uniform criteria hinders their use.

2.6 Prevalence and incidence of occupational rhinitis

There is very limited data on the statistics of occupational diseases available from Finland before occupational diseases have been compiled since 1964. It has been possible to study the risk of various occupations for OR from those statistics (Hytönen *et al.*, 1997). Occupations with a high level of dust, as well as sensitizing exposure levels in the air can be considered as risk areas for OR (Hytönen *et al.*, 1997, Riu, *et al.* 2007).

The methods used for the definition (questionnaire, objective evaluations), baseline characteristics of population studied (like age and atopy) exposure time, type and exposure concentration in the air all affect to the results of OR prevalence and incidence. Most epidemiologic studies are done with symptom questionnaires, some also with specific IgE measurements, and a few studies have used challenge tests for confirming the causative role of the suspected occupational agent. The prevalence of OR may be overestimated or underestimated (healthy worker effect), making analysis and comparison of results difficult. An extensive review of the prevalence and determinants of occupational rhinitis has been published some years ago (Siracusa *et al.*, 2000). The prevalence of OR in occupations with HMW exposure varied from 2% to 87% and with LMW agents from 3 to 48%. Incidence of OR has been reviewed to be between 0.3 to 13.1 x100 person years (Moscato *et al.*, 2009). Some more recent incidence/prevalence studies not included in those reviews are collected to a Table 1.

Table 1. *Some recent incidence /prevalence studies of OR. Studies that have been confirmed with a specific IgE tests or challenge tests are marked with*.*

Reference/ agent	**Subjects** (n)	**Mean age** (years)	**Duration of follow-up** (years)	**Incidence of OR** (.100 person years)	**Prevalence of OR symptoms and sensitization**	**Prevalence of OR confirmed with challenge test**
(Ruoppi *et al.*, 2004)/ laboratory animals & laboratory animal workers	156	34				6%* of responders (response rate 64%)
(Gautrin *et al.*, 2008) laboratory animals, flours, latex	408		8	1.7		
(Folletti *et al.*, 2008) laboratory animal workers (review)	1175	33			2.9-18.8%	
(Walusiak *et al.*, 2004) apprentice bakers	287	16	2	8.4-12.5%		

There are only limited data available on the longitudinal incidence of occupational respiratory diseases. In the report of Folletti et al. it was suggested that occupational allergic asthma due to laboratory animals has been declined progressively from 1976 to 2001 from 8.2% to 4.2%, and it may be due to the reduction of the exposure (Folletti *et al.*, 2008). They concluded that OR is three times more frequent than occupational asthma and that it seems that further reduction of the exposure would be needed in to prevent the onset of OR.

In Finland, the reports of allergic OR reported to the Register of Occupational Diseases has declined from the end of 80's 400 cases/ year to around 100-150 until the end of 2003, and has not increased from that since then. There is decrease in all the main causative agents: flours, animals, mites, molds and wood dusts (Vaaranen *et al.* 1989, Karjalainen *et al.*, 1996, Karjalainen *et al.*, 1998a, Karjalainen *et al.*, 1998b, Karjalainen *et al.*, 2001b; a; Karjalainen *et al.*, 2002; Riihimäki *et al.*, 2003; Laakkonen *et al.*, 2007; Karjalainen *et al.*, 2008). As there have been changes in the registration and notification practices Finnish Register of Occupational Diseases, the statistics from 2005 on are not fully comparable to the older reports (Karjalainen *et al.*, 2008).

2.7 Health-related quality of life in occupational rhinitis

Definitions

The Health-related quality of life (HRQoL) questionnaires indicate self-assessed impact of ill-health and diseases on functioning and well-being (Laaksonen *et al.*, 2006). Various questionnaires can be used to measure cross-sectional differences in quality of life between patients at a point in time (discriminative instruments) or longitudinal changes in HRQoL within patients during a period of time (evaluative instruments). Both discriminative and evaluative instruments must be valid (really measuring what they are supposed to measure) and have a high test-retest reliability and responsiveness. Two basic approaches to quality of life measurement are available: generic instruments that provide a summary of HRQoL and specific instruments that focus on problems associated with single disease states, patient groups, or areas of function. Generic instruments include health profiles and instruments that generate health utilities (Guyatt *et al.*, 1993).

Rhinitis-specific quality of life questionnaires

There are several disease-specific instruments designed for the evaluation of rhinitis, like the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) (Juniper *et al.*, 1999), the mini Rhinoconjunctivitis Quality of Life Questionnaire (Anderson *et al.*, 1999; Juniper *et al.*, 2000) and the Sinonasal Outcome Test -16 (SNOT-16) (Anderson *et al.*, 1999). However, as OR has a mostly allergic origin, the questionnaires addressing AR seem to be more relevant than the questionnaires addressing sinusitis and/or nasal polyposis. Because asthma and rhinitis often coexist, a specific Rhinasthma questionnaire has been designed to cover the rhinoconjunctivitis-asthma HRQoL comorbidity (Baiardini *et al.*, 2003). As most allergic patients experience symptoms from multiple organs, disease-specific HRQoL measures used alone may be deficient. In a study with the rhinoconjunctivitis specific questionnaire RQLQ and generic questionnaire 15D, the general HRQoL instrument scores generated a more comprehensive view of the impact of allergen exposure on a patient's quality of life than RQLQ (Petersen *et al.*, 2008).

General health-related quality of life questionnaires

Correlations between conventional nasal symptom-severity diaries and health-related quality of life have been found to be only weak to moderate. Therefore, to get an overall picture of a patient's health status, it is essential to measure quality of life. General health-status questionnaires are able to compare burden of illness across different medical conditions. The most commonly used and the most validated generic instruments are the Sickness Impact Profile, the SF-36, and the Nottingham Health Profile (Juniper, 1997). The SF-36 is the most commonly used in the English language literature, and it is also

distributed with names RAND-36 Item Health Survey 1.0 and RAND-36 Health Status Inventory, with minor differences in scoring (Coons *et al.*, 2000). RAND-36 is also freely available and validated to the Finnish population as well as to various other cultures. In the health economic studies, however, preference-based HRQoL measures can be used that produce a single summary score covering the whole HRQoL for the comparison of the impact of various diseases to each other (Räsänen *et al.*, 2006). Some examples of them are EQ-5D, Assessment of Quality of Life (AQoL) and 15D (Sintonen, 2001).

Quality of life in occupational rhinitis

Allergic rhinitis is known to impair HRQoL (Bousquet *et al.*, 1994; Larsson *et al.*, 2007). It is known that AR generally impairs the well-being of the patients, and that it affects social life, sleep, school and work. In a study with seasonal (SAR) or perennial allergic rhinitis (PAR) from adult population from England, 22% of PAR sufferers and 11% of SAR sufferers described their condition as annoying or disrupting to everyday life, and ≥ 33% of all patients reported their symptoms occasionally or frequently affecting their home or social life (Scadding *et al.*, 2000).

The HRQoL has been studied in an occupational setting only in a minor group of the patients, the greenhouse workers (Groenewoud et al., 2006). In that study sensitization to bell pepper pollen had a significant negative effect on rhinitis specific quality of life scores. The other allergens had no effect on quality of life. Greenhouse employees scored higher on limitations in activities and much lower on emotional, sleeping, and practical problems, compared with individuals with perennial rhinitis. The general HRQoL questionnaires among OR patients has not previously been studied.

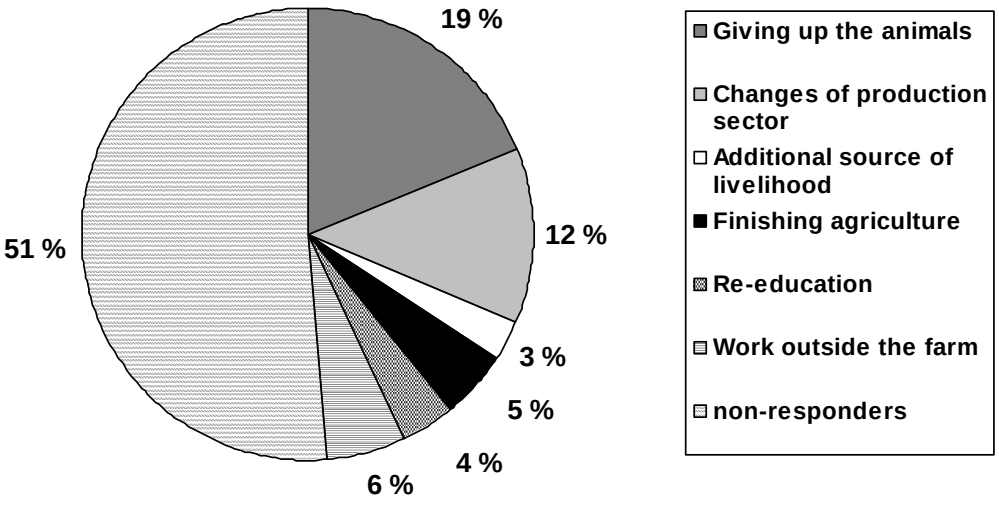
Socioeconomic effect of occupational rhinitis

The economic impact of OR has been sparsely studied. In general, the cost-of-illness studies separate the cost of the disease to those associated to the medical care treatment and to the indirect costs. Direct costs relating to AR are comprised of medical costs, physician visits and hospitalizations. AR causes indirect costs relating to the productivity loss and days lost from work. In rhinitis patients, self-reported effectiveness while working -assessed by a telephone survey-, has been found reduced in 44% of all kinds of rhinitics (Blanc *et al.*, 2001). Indirect costs of AR arising out of productivity loss are more important than direct healthcare costs (Simoens and Laekeman, 2009). Also, worsening of associated airway diseases (sinusitis or asthma) may cause indirect costs in relation to AR (Ray *et al.*, 1999; Yawn *et al.*, 1999; Price *et al.*, 2005). In one recent study the work productivity was negatively affected by some specific symptoms of AR (i.e. sneezing, nasal congestion, and watery eyes), sleep disturbance, and general health related quality of life (Szeinbach et al., 2007).

The interventions of OR aim to reduction or cessation of the exposure to the causative agent. If the exposure load cannot reasonably be avoided or reduced in the workplace, re-education due to occupational diseases is discretionarily compensated by mandatory insurances in Finland. The influence of total avoidance of exposure in occupational asthma (OA) has been stated to be associated with substantial socio-economical consequences in Italy, where system did not guarantee prompt and automatic compensation for subjects with OA (Moscato *et al.*, 1999). Of subjects with OA, 25–38% suffers from prolonged work disruption, and 42–78% reports a substantial loss of income. Financial consequences of OA are consistently more pronounced in workers who avoid further exposure to the causal agent (Vandenplas *et al.*, 2003).

A recent questionnaire study among farmers in Finland has studied the effect on the occupational AR to their work ability (Järvenpää and Kulhua, 2008). In the questionnaire study, it was researched how the farmers have managed with AR and how it has affected their farming. Of the respondents, 19.6% could continue their work normally, 54.7% were able to continue their work with a mechanical full-face respirator and 14% had to stop working as farmers (Järvenpää and Kulhua, 2008). In addition, most of the respondents needed to make changes and adapt the farming livelihood to the situation (Figure 3). The costs of acquisition and cost of the use of motorized helmet respirators among farmers had been previously compensated by the insurance only if the patients have been shown to have OR and has bronchial hyperreactivity. Since the study showed their usefulness, they have become compensated also without hyperreactivity.

Figure 3. *The socioeconomic effect of allergic OR among farmers in Finland (Adapted from Järvenpää and Kulhua, 2008)***.**



Risk of asthma and need of medication in occupational rhinitis

Rhinitis and asthma are known to be closely connected diseases. The majority of patients with occupational asthma (OA) also suffer from OR, which most often precedes the development of OA (Moscato *et al.*, 2009). Allergic rhinitis in general increases the risk of asthma by about three times among atopics and non-atopics and by more than five times among the highest IgE group (Guerra et al., 2002). The situation is quite similar in OR: in Finnish patients with confirmed occupational rhinitis the crude risk rate of asthma was 4.8, 5.4 for those with occupational rhinitis accepted for compensation, and 3.7 for patients with unaccepted occupational rhinitis. The risk ratio varied according to occupation and was the highest among farmers and wood workers, both groups having a sevenfold risk (Karjalainen et al., 2003). Additional risk factors for asthma have been found to be bronchial hyperresponsiveness, and both severity and duration of rhinitis (Moscato *et al.*, 2009). When the potential risk factors for occupational asthma were compared among apprentices exposed to high-molecular weight allergens, the bronchial hyperresponsiveness was the most significant one, while rhinoconjunctivitis (defined as at least one symptom of rhinitis or conjunctivitis), atopy (defined as two or more ≥ 3 mm wheals in SPT) and bronchial hyperresponsiveness (provocative concentration to methacholine causing a change in FEV1 of 20% (PC20) of ≤ 16 mg/ml) were compared (Gautrin et al., 2003).

Among prospective cohorts of an 8-year follow-up of 408 apprentices entering programs involving exposure to high-molecular-weight allergens and who held a job related to their training (78%), the incidence of sensitization, rhinoconjunctival and chest symptoms, and bronchial hyperresponsiveness at follow-up was 1.3, 1.7, 0.7, and 2.0 per 100 person-years, respectively. A high proportion of subjects in a job not related to training experienced a remission of symptoms acquired during apprenticeship (Gautrin *et al.*, 2008).

Among the Finnish farmers with OR, the prevalence of asthma has been in a questionnaire study 26.8% (with response percentage of 53%), (Järvenpää and Kulhua, 2008). The medication needed for OR has been rarely addressed. In the Finnish study among farmers with OR, 52.5% regularly used medication for their rhinitis, 44.7 % did not use medication and 2.7% did not respond (Järvenpää and Kulhua, 2008).

2.8 Proteomic methods in allergen studies

"Proteomics" is the large-scale screening of all proteins of a cell, organism or biological fluid; a process which needs stringently controlled steps of sample preparation, 2-D electrophoresis, image detection and analysis, spot identification and database searches (Görg, 1998). Specific allergens can be identified in order to find out the specific reason

for the IgE-mediated allergic symptoms of the patients, for the standardization and development of the allergy tests, immunotherapy agents and hypoallergenic plants and products. The biochemical methods needed and used here for detection and analysis of proteins from biological samples are described briefly.

Chromatography

Chromatography is the collective term for a family of laboratory techniques for the separation of mixtures. Preparative chromatography separates the components of a mixture for further use and is thus a form of purification. There are numerous techniques of chromatography, and they can be divided e.g. by the mechanism of separation (Lewis, 2006).

In exclusion chromatography, separation is based mainly on exclusion effects, such as differences in molecular size and/or shape or in charge. The term size-exclusion chromatography may also be used when separation is based on molecular size. The term gel filtration chromatography can be used to describe the process when the molecules in solution are separated according to differences in their sizes as they pass through a column packed with a chromatographic medium, which is a gel (GE Healthcare Bio-Sciences AB, 2002).

Chromatographic techniques are often classified by specifying the physical state of both phases used. Accordingly, in liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. Present-day liquid chromatography generally utilizing very small particles and a relatively high inlet pressure, is often characterized by the term high-performance (or high-pressure) liquid chromatography (HPLC). In reversed-phase HPLC, the mobile phase is significantly more polar than the stationary phase, e.g., a microporous silica-based material with chemically bonded alkyl chains (Sandra *et al.*, 2008).

Protein immunoblot, SDS-PAGE and electrophoresis

Protein immunoblot (also called western blot) allow investigators to define the molecular weight (MW) of a protein and to measure relative amounts of the protein present in different samples. Proteins (or their fractions) can be separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to their MW (Gorg *et al.*, 1988; Berkelman and Stenstedt, 2002). SDS-PAGE maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure. The proteins are then moved to nitrocellulose (or other) membrane by electrophoresis and the IgE immunological detection can be done with patient sera after incubating with a general protein (like milk). During the detection process, the membrane is probed for the protein of interest with a modified antibody

which is linked to a reporter enzyme, which when exposed to an appropriate substrate drives a colour reaction.

Mass spectrometry and bioinformatics tools

The fingerprints of peptides of IgE binding proteins can be generated using matrix-assisted laser desorption ionization (MALDI) tandem time-of-flight (TOF) mass spectrometry (MS) and identifying the proteins with the commercial databases (Akagawa *et al.*, 2007). Information technology is employed in gathering, storage, retrieval and analysis of these data. At least a dozen important allergen databases and data repositories have been developed to date (Brusic *et al.*, 2003). These data are analysed using general and specialist bioinformatics tools. The major applications of bioinformatics include support for allergen characterization, assessment of allergenity, and identification of allergic cross-reactivity (Zhang et al., 2006).

3. Aims of the study

The aim to the present thesis was to assess the diagnostics of OR in FIOH. In addition, we wanted to evaluate the long-term quality of life among OR patients in Finland as well as to study the allergens of wheat grain, the main cause of OR in Finland.

The specific aims of the thesis were:

I To evaluate the usefulness and clinical value of the nasal provocation tests with various allergens, haptens and non-IgE-mediated irritants in the diagnostics assessing occupational rhinitis.

II To study the outcome of occupational inhalation challenge test in assessing occupational rhinitis in patients with a suspicion of both upper and lower airway occupational symptoms.

III To evaluate the health-related quality of life among allergic occupational rhinitis patients. As a control group, a random population of similar age and locality with and without doctor diagnosed allergic rhinitis, was studied.

IV To characterize relevant wheat flour allergens among Finnish patients with baker's rhinitis or asthma and determine their clinical relevance with skin prick tests.

4. Subjects and methods

4.1 Patients (I-IV)

Altogether 1495 outpatient consultations were done in Finnish Institute of Occupational Health (FIOH) at the unit of Otorhinolaryngology in 2001-2003 because of suspected OR. Of these, 11 % (165 patients: 117 women and 48 men) were examined by a nasal provocation test (NPT) and were the subjects of the study I. The average age of these patients was 41 years (range 23-64 years). The inclusion criteria for performing the NPT were: 1) obvious exposure to a known occupational allergen or mould damage found in the workplace, 2) symptoms referring to work-related allergic rhinitis, 3) no other obvious cause for the rhinitis and 4) no contraindications for the NPT. The symptoms suggestive of occupational AR were episodic, work-related occurrence of nasal obstruction, rhinorrhea, sneezing, itching of the nose and/or postnasal drainage. In addition, sensitization against work-related allergen(s) was generally found, supporting the suspicion of occupational disease.

Altogether, 3895 otolaryngology consultations were performed at FIOH in 1997–2003 because of suspected upper airway disease. In cases where the suspected allergen was either chemical or the patient had both upper and lower airway symptoms related to work, the patients, 21% (829) in all, were examined by an inhalation challenge test. Those patients were the subject of the study II. The age of these patients varied between 18 and 65 years with a mean age of 44 years.

The subjects of the study III were 212 consecutive patients with occupational rhinitis (OR) to a protein allergen, gathered from the patient registry of the FIOH. Their inclusion criteria were 1) diagnosis of occupational AR to a protein allergen, 2) a visit to the clinic during 1991-2003 and 3) no asthma found in examinations carried out at the same time as OR diagnosis. Of the OR diagnoses, 93-97% had been verified by challenge tests. We had no access to the diagnostic case records of five (4%) patients, because they had been diagnosed in other hospitals. In addition, 414 controls, randomly chosen from the Finnish population registry, with the same age and locality, were asked to complete the questionnaires. For the controls, a positive answer to doctor-diagnosed AR was considered to indicate AR. This question has been validated and found to have specificity of 0.93 and sensitivity of 0.52 among Finnish young adults as compared to SPT /specific IgE confirmed AR (Kilpeläinen et al., 2001).

The subjects of the study IV were 20 Finnish patients diagnosed with baker's rhinitis or baker's asthma between 4th February 2004 and 31st December 2007, who had subjective symptoms in relation to wheat exposure (Table 1). In addition to that, our inclusion criteria included specific IgE antibodies ≥ 0.35 to wheat flour (ImmunoCAP, Phadia, Uppsala, Sweden). During the diagnosis of baker's rhinitis or asthma, 19 out of 20 had

positive (3 mm or larger) skin prick prick test wheals to wheat flour (100 mg/ml mixed with 0.1 M PBS pH 7.4). In addition, 19 out of 20 of the diagnoses of bakers' rhinitis and/or asthma had been verified with a nasal provocation test or inhalation challenge tests with grains the patients were exposed to at their workplaces. Control group consisted of 10 healthy subjects, with negative SPT reactions to wheat and common environmental allergens tested.

Ethical considerations

The study designs were approved by the local ethics committee. All clinical subjects participated voluntarily, and gave their written, informed consent.

4.2 Methods

Challenge test methods (I, II)

In study I, the NPTs were performed like previously described (Hytönen and Sala, 1996), using the scoring criteria counting changes in rhinorrhea and mucosal blockage as the measure of positivity. Liquid allergens were applied on the topical surface of the inferior conchae, usually by applying a 0.1 ml allergen-saturated small piece of neurosurgical cotton disk (Cameco, Sweden) or, to avoid any irritation, by dropping 0.1 ml of allergen solution from a syringe with a metal suction head. The dry provocation agents were applied with the head of a nasal elevator on the topical surface of both inferior conchae. Changes in the rhinorrhea and blockage from both nostrils were scored from 0 to 3 points (from dry mucosa or mucosa lining only the bony conchae to dripping mucus or swelling of the mucosa obstructing nasal cavity). The reactions scored with 4 points or more in 30 min time were considered as confirmed rhinitis reactions to the test agent, if the control challenge test with placebo did not produce reactions. The test agents were commercial allergen test agents, if such were available. Otherwise we used the patient’s own workplace agents or pure ingredients of them to the challenge tests.

In study II with the inhalation challenge tests the concentrations of the challenge agents were adjusted to mimic the exposure situation in the workplace and to remain below the Finnish OELs, if existing, to avoid irritant reactions. Challenge agents were used either as such, mixed in lactose powder, or diluted if serious reactions were suspected based on medical history or SPT reactions. The inhalation challenge tests usually lasted 30-45 min. depending on the agent and patient reaction, but were finished earlier in cases of evident reactions.

Compared with local nasal challenges with type 1 immediate reactions, the evaluation of pulmonary reactions requires a follow-up of at least 6 hours after the exposure, because the dual and late reactions appear frequently in lower airways. In our clinic, the subjects are admitted as inpatients for the tests and the examinations usually last for 1–2 weeks, while local nasal challenges are typically done in 2 days.

Challenge test criteria: nasal secretion and placebo reactions

In the study I, we compared the diagnostic criteria used in FIOH with nasal scoring (Hytönen and Sala, 1996) and the amount of nasal secretion of 0.2g. The nasal secretion dripping out of the nose was collected in 30 min time to a pre-weighted plastic mug, and in the end of the follow-up period, the secretion from anterior to nasal skin-mucosa junction was suctioned in the anterior rhinoscopy to a pre-weighted suction tip (with tip size 2 mm) with a collector, if needed. The total nasal secretion was weighted. In cases which the amount of secretion was scored normal (1) or dry (0), with no change of scoring points from rhinorrhea, the amount of secretion was valued in this analysis as zero. The number and percentage of provocation tests with positive reactions with the occupational agents and the control agents were evaluated.

In the study II, provocation tests in occupational inhalation challenges concerning rhinitis were done according to the international guidelines of bronchial challenge tests (Moscato *et al.*, 2003; Vandenplas *et al.*, 2006), except that the patients were instructed to breathe through the nose and through the mouth on alternate breaths. In the case of commercial allergens, the Spira facial mask (Laerdal Medical, Stavanger, Norway) was used, covering both the nose and the mouth.

Classification of the provocation test agents

The challenge test agents were classified to organic and inorganic agents, with the type of the most-likely allergen or agent provoking the OR reaction. We analyzed the number of IgE-mediated and positive reactions in the provoked rhinitis reactions in each group of the provocation agents used.

Questionnaires to measure health-related quality of life (III)

The RAND-36 Item Health Survey 1.0 (RAND-36) (Hays *et al.*, 1993) was chosen for the assessment of general HRQoL as it is known among the most common questionnaires available and it is well validated. The Rhinasthma questions were chosen for the evaluation of disease-specific HRQoL as it is designed to measure rhinitis-asthma comorbidity.

For possible influence to HRQoL, we analyzed level of education, employment over the last 12 months, current rate of smoking, number of years of smoking, diagnosis of asthma made by a doctor and use of rhinitis medication during the last year.

Statistics

In the paper III, the differences of the means between the patients and the controls were tested using one-way nonparametric Kruskal-Wallis analysis of variance. The Mantel-Haenzel chi-square test was used with chi-square tables. Correlations between the Rhinasthma and RAND-36 scores were analyzed using Spearman correlation coefficients. The differences between HRQoL of differently exposed groups of patients and controls were compared using Wilcoxon two-sample tests, and all occupational protein allergens causing OR were considered to constitute one group in the analyses.

Wheat protein extraction, purification and skin prick tests (IV)

Water-soluble wheat (*Triticum aestivum*, cultivar Tjalve) proteins were extracted from grounded grains in ammonium bicarbonate or ethanolamine with constant stirring for 4 hours. After centrifugation supernatants were collected, filtered and frozen as aliquots. Protein assays were made. Ammonium bicarbonate extracts were used to analyze IgE reactivity of wheat water-soluble proteins by immunoblotting after 2-dimensional (2D) sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and ethanolamine extracts were used for isolation of water-soluble proteins from wheat flour.

Purification of proteins from ethanolamine extracts involved gel filtration and reversed-phase chromatography. The fractions containing certain proteins (identified by tandem-MS and shown to be allergenic by immunoblotting) were pooled, concentrated and further purified by another gel-filtration step. The final purification of different protein pools was performed by reversed-phase chromatography.

Identification and characterization of wheat allergens

Water soluble proteins from wheat ammonium bicarbonate extracts were separated by means of electrophoresis 2D SDS-PAGE and analyzed by immunoblotting to detect proteins that bind IgE in the sera of patients with baker's rhinitis and asthma. Purification of wheat allergens was monitored by 1-dimensional (1D) SDS-PAGE and immunoblotting. For molecular characterization of proteins, the molecular mass and peptide mass fingerprint analyses were performed by MALDI-TOF-MS analyses.

1- and 2-dimensional sodium-dodecyl-sulphate polyacrylamide gel electrophoresis

Protein samples were evaporated and reducing buffer was added for 1D SDS-PAGE. To perform 2D-SDS-PAGE, crude wheat extract was rehydrated and the mixture was absorbed into immobilized pH gradient strips. Isoelectric focusing was performed after which proteins were separated with 12 % SDS-PAGE according to the manufacturer's instructions (BioRad). After 2D SDS-PAGE, one of the parallel gels was stained and the other one was exposed to immunoblotting with sera from baker's rhinitis and asthma patients.

Immunodetection for wheat allergens

Immunoblotting was performed as described previously with modifications (Palosuo *et al.*, 2001a). Briefly, proteins were transformed by electrophoresis (MiniTransBlot, BioRad) from SDS-PAGE gels onto polyvinylidene fluoride membranes. After that membranes were incubated with diluted patient's sera. Biotinylated goat anti-human-IgE was added, followed by streptavidin-conjugated alkaline phosphatase and the colour development solution.

Tandem mass-spectrometry

Protein spots were cut out from the gels, reduced and alkylated before in-gel digestion with modified sequencing grade porcine trypsin (Promega, Madison, Wisc). After removal of the supernatants, the remaining peptides in gel pieces were extracted twice. Combined peptide extracts were dried in a vacuum centrifuge. Each peptide mixture was analyzed by automated LC–MS/MS coupled to an electronspray ionisation quadrupole TOF MS. Reversed phase separation of peptides was carried out. Peptides were eluted from the column with a linear gradient.

The obtained mass fragment spectra were searched in the non-redundant protein database compiled by the National Center for Biotechnology Information against all entries using in-house Mascot v.2.1 (Matrix Science Ltd., London, UK) with the following parameters: trypsin specificity; one missed cleavage; precursor ion mass accuracy of 0.2 Da and 0.5 Da mass tolerance for fragment ions; fixed modification was carbamidomethylation of cysteine and oxidation of methionine, histidine and tryptophan were accepted as variable modification (Chamrad *et al.*, 2004).

Skin prick tests

Skin prick tests (SPT)s were done according to the general guidelines (Mailing, 1993). The suspected occupational aeroallergens were tested by a SPT, skin prick-prick test,

specific serum IgE test, or both. Purified wheat allergen proteins (concentrations 5, 50 and 500 μg/ml) were used in SPTs. Skin wheals ≥ 3 mm were considered to show sensitization to a purified wheat allergen (Juntunen-Backman *et al.*, 1995). Histamine 10 mg/ml was used as a positive control and commercial positive control, and PBS partly together with a Coca solution (sodium chloride 0.5%, phenol 0.4%, and sodium hydrogen carbonate 0.3% diluted to aqua) and 20 mM ethanolamine as negative controls.

5. Results

5.1 Provocation test studies (I-II)

The nasal provocation tests (I) were positive in 39% of the occupational allergen provocations, whereas in 10% (19/193), placebo tests gave a positive provocation reaction. Forty-seven percent (78/165) of the patients were diagnosed as having OR. On average, three NPTs were performed on each patient, i.e. mainly one control and two allergen provocations. High-molecular-weight allergens caused most of the positive reactions in our patients. The flours (68% positive), and indoor plants and flowers (73%) were most likely positive in the provocation test, followed by animal allergens (37%). Wood dusts gave 50% positive NPT, mostly without IgE-sensitization. Atopic propensity was found in 90% of the patients reacting positively to dry wood dusts in our material. The NPTs performed towards molds were only seldom positive (18%), and only a minority of those patients had IgE towards molds (9%) or atopic propensity in skin prick tests (34%).

Nasal secretion was measured or judged in most NPTs (84%), to test the parameter in the diagnosis of OR. In positive NPTs the nasal secretion varied between 0.1 and 11.3g, $111/117 \geq 0.2$g. In all but one provocation secretion < 0.2 g (302/303) was regarded as no OR, so the measured secretion matched well with scoring. No generalized allergy reactions occurred in the provocation tests.

Of the nasal provocation tests, 37% were control provocations and 10% (19/189) out of them were positive. Among the patients with positive control tests agent reactions, some elicited similar rhinitis reactions as the occupational agents. Among these cases, there were some patients suffering from nasal polyposis and patients who had challenge tests done with glycerol-containing agents. Mostly, the repetition of the nasal challenge test did clarify the situation, especially if temporary hyperactivities of the nose were withdrawn.

In inhalation challenge tests (ICT)s (II) evaluated, an average of three challenges were performed on each patient. Rhinitis appeared in 13% (111/829) of the challenged patients, while asthmatic reactions appeared in 25% (211/829) of these patients. From the inhalation challenge tests 10% produced positive rhinitis reaction and 13% of the tests positive asthma reaction. Organic agents were the most frequent causes of OR in our patients and, the causes of them were quite similar to the NPT.

Inorganic causes causing rhinitis reactions in the inhalation challenge tests are shown in the Table 2.

Table 2*: Inorganic agents causing rhinitis in the inhalation challenge tests*

Occupational agent	Positive challenge tests with rhinitis	Occupational source
Acid anhydrides	14	hardeners in epoxy resin systems, welding of painted metal
Formaldehyde and glutaraldehyde	11	chemistry reagents, particleboards, cloths, fixatives, machining fluid (main suspected agent)
Persulphates, thioglycolates and paraphenylenediamine	13	hairdresser's chemicals
Methacrylates and acrylates	7	tooth fillings, adhesive glues and hair sprays
Di-isocyanates	3	paint hardeners and wood coatings
Welding fumes	2	welding
Chloramine-T	2	disinfectant in cleaning work
Colophony fumes	1	soldering
PVC fumes	1	PVC heating
Epoxy	1	glue
Aziridine (ethylene imine)	2	leather finishing work

Inhalation challenge tests with a placebo agent produced rhinitis reactions in 2% of the challenges in our study, and 10% of the occupational challenges triggered rhinitis reactions that were considered as OR. In the NPT, we have previously described a higher proportion of rhinitis reactions, both from occupational (39%) and placebo challenges (10%). All nasal challenges were done without any serious reactions. No anaphylactic reactions have appeared in our institute in the ICTs.

5.2 Health-related quality of life study (III)

Sixty-two percent (62%) of the OR patients, and forty-four percent (42%) of the controls completed the questionnaire. The occupational allergens in the respondents' and non-respondents' groups were similar, but the respondents were on average 3.5 years older than the non-responders (45 and 49 years, $p<0.0001$), and women answered more often than men did (69% and 55%, $p<0.0001$).

During the questionnaire, 22% of the OR patients were no longer exposed to the occupational agent that had caused their OR. Fifty-eight percent of the patients estimated that their exposure had decreased, and 20% felt having similar or increased occupational allergen exposure compared to the time of their OR diagnosis.

The OR patients suffered from perennial rhinitis symptoms significantly more often (26%) than the age and sex matched control population (18%), while the controls reported more seasonal rhinitis, compared to the patients (41% versus 20%, p=0.0002). A total of 20% of the patients in our study used rhinitis medications regularly, 53% periodically or temporarily, and 27% not at all. Only 10% of the controls who answered used regular rhinitis medication, 47% periodically or temporarily, and 43% not at all (p=0.001). The controls and patients results in the subgroups are shown in Table 3 below.

Table 3*: Frequency of using rhinitis medication among patients with occupational rhinitis compared to controls with or without AR* (complementary findings by Airaksinen et al, unpublished results)

Frequency of rhinitis medication use	**Healthy Controls** % of the answers	**Patients (with no exposure- low exposure- most exposed group)** % of the answers	**Controls with AR** % of the answers
Regularly	2	8-15-52	32
Periodically or temporarily	45	52-60-34	53
Not at all	53	40-25-14	15

The types of medication were mainly corticosteroids and antihistamines (Table 4) in our responders, but the response to this question was low (23-42% of all patients) and therefore these results need to be considered as suggestive only.

Rhinitis medications used during last year	**none** % of the answers	**<10 days** % of the answers	**10-59 days** % of the answers	**2-6 months** % of the answers	**6-9 months** % of the answers	**9-12 months** % of the answers
Antihistamines	23	21	22	11	8	15
Corticosteroid-sprays	39	8	20	6	7	20
Leukotriene antagonists	55	18	18	6	2	2

Table 4*: Types of rhinitis medications used among patients with occupational rhinitis years after their diagnosis (complementary findings by Airaksinen et al, unpublished results)*

The work attendance of the patients and the controls did not differ. A total of 17% of our patients had been re-educated because of OR. Out of the re-educated patients, 9% reported increase, 33% decrease, and 58% no changes in livelihood due to OR.

As we grouped the patients according to their current occupational allergen exposure the respiratory HRQoL of the patients no longer exposed to occupational allergens did not differ from the healthy controls. Occupational allergen exposure that had remained similar

or increased since the time of OR diagnosis, lowered the patients' respiratory HRQoL to the same extent as did the diagnosed AR among the controls.

The non-exposed patients had no differences to the healthy controls except in one RAND-36 score. The bodily pain score indicated an even higher HRQoL among the non-exposed OR patients than among the healthy controls. Only the general health score was statistically lower among the exposed OR patients compared to non-occupational AR.

Determinants of HRQoL in our study found were reduced livelihood due to OR, frequency of rhinitis medication usage, and asthma which was related to deteriorated HRQoL both in Rhinasthma and RAND-36. Education did not influence any Rhinasthma scores, but did affect the RAND-36 general health score ($p<0.01$), physical functioning score ($p<0.0001$) and physical role scores ($p<0.05$). Age did not affect the Rhinasthma scores, but did decrease five out of eight RAND-36 scores. The type of occupational allergen did not have influence on the results of Rhinasthma or RAND-36.

5.3 Occupational respiratory wheat allergy (IV)

Water-soluble extract of wheat grains is protein-rich and Western blotting with sera of baker's rhinitis and asthma patients indicated that many of these proteins among our patients bind IgE, i.e., are allergens. However, IgE-binding responses of individual patients to these proteins varied. Tandem MS analysis of IgE-binding protein bands suggested that especially alpha-amylase inhibitor α-AI (MW = 12-17 kDa), lipid transfer proteins LTP 1 (MW = 12 kDa) and nsLTP2G (MW = 7 kDa), thaumatin like protein (TLP) (MW = 23 kDa), 1-Cys-peroxiredoxin (PER1) (MW = 24 kDa), glutathione S-transferase (GST) (MW = 25 kDa), 27K protein, xylanase inhibitor protein 1 (MW = 34 kDa) and peroxidase I (MW = 39 kDa), are important allergens among our baker's rhinitis and baker's asthma patients.

Two gel filtration steps and reverse-phase chromatography were used to separate IgE reactive water-soluble wheat proteins from each other. We focussed to purify LTP, α-AI, PI and TLP. In SPTs, all our wheat respiratory allergy patients had positive SPT to a total wheat flour preparation and to the pool of water-soluble wheat proteins. Dose-dependent SPT responses to purified water-soluble wheat allergens were found and 75% of our patients (15/20) recognized at least one tested wheat allergen in SPT, on average 2/5 (from 0/5 to 5/5). Sixty percent, 60% of the patients recognized α-amylase inhibitor as an allergen, 45% recognized TLP and PI, and 30% recognized LTP as an allergen. All ten control subjects had negative SPT reactions to tested wheat allergens.

6. Discussion

6.1 Provocation test studies (I, II): aetiology, diagnostics and classification

Nearly half of the patients with upper respiratory related rhinitis symptoms did receive a diagnosis of occupational disease in NPTs (47% of the patients had positive provocation test), but in the inhalation challenge tests it was quite rare finding (only 13% of the patients had a rhinitis positive challenge test). The patients who did not receive OR diagnosis, were likely to have unspecific hyperreactivity of the nose, work-exacerbated rhinitis, mild rhinitis reactions that did not reach the diagnostic level, or some other form of concurrent rhinitis or upper airway irritations.

The patient material in this thesis was highly selected. According to the medical history, immunological tests, clinical examination and related studies, the otolaryngologist selected 11% of the outpatient consultation patients with work-related rhinitis symptoms for nasal provocation test studies. To the inhalation challenge test came 21% of the patients visiting FIOH otolaryngologist. Work-related rhinitis and simultaneous bronchial work-related symptoms were studied with otolaryngologist follow-ups in these inhalation challenge tests. The patients of this thesis, studied with provocation tests (I, II), had increased amount of atopy (in study I 47% and study II 37%) compared to general population (34% during the time of the studies). The increased amount of atopy among the patients studied may partly explain the quite high percentage of nasal hyperreactivity reactions found in the placebo tests in the study I (10%) compared to the study II (2%). The causative agents suspected, patient materials and the provocation tests were different in studies I and II, also affecting to the results.

The nasal provocation test scoring criteria used in FIOH for NPT positivity evaluation was compared to nasal secretion amount of 0.2 g. We derived it from one unilateral NPT method for nasal provocation test evaluation (Pirilä and Nuutinen 1998) by multiplication of cumulative nasal secretion of 30 min time by two to 0.2 g. Our patient material was retrospective in nature, heterogeneous and there were numerous causative agents and many allergen and occupational materials in it. These facts result in wide normal variations for the test results. The study of Pirilä and Nuutinen was a prospective work with one allergen, homogenous patient materials with selected controls and several methods of evaluation of NPT. Taking these limitations into account, and comparing the results, most of the scoring positive NPT results of FIOH had positive nasal secretion criteria $\geq$ 0.2 g (111/117 provocations), and all cases with positive NPT had at least 0.1 g secretion amount. The result favours that the two different methods of nasal provocation tests criteria give quite similar nasal secretion results in magnitude. It is also in favour that

nasal secretion amount is useful, easily used and good criteria in the nasal provocation tests and in comparisons of various challenge test method results.

The nasal and inhalation challenge test methods differ in several ways, but we used the same rhinitis criteria in their evaluations. In nasal challenges, most of the tested agents are IgE-mediated allergens, and the typical allergic rhinitis reactions are usually quite strong and easy to recognize, if the allergens used and their concentrations are adequate. In specific NPT, the local concentration of the allergen used becomes higher than during the inhalation challenge test, without the fear of serious reactions. As the NPT is searching for IgE-mediated allergy, the results of sensitization can be used mostly to the selection of the patients to the provocation tests. In ICT the non-IgE mediated reactions are more common, and the non-IgE-mediated reactions are not so well understood, or characterized, and therefore the patient selection to the challenge tests is more challenging. Inhalation challenges with LMW (generally inorganic agents) have been reported to produce less intensive rhinitis than the ones with HMW (generally organic protein challenges) (Malo *et al.*, 1997), although the prevalence of the symptoms do not differ in HMW and LMW agents. This may relate to a fact that HMW agents generally exist as dry or liquid aerosol, whereas LMW agents are more generally vapours. The aerosols may more readily be deposited in the upper airways and cause symptoms there.

NPT was safe and well-tolerated in our patients. No serious reactions have occurred in these provocation test series. In the ICTs it has been estimated that chamber challenge reactions may cause asthmatic symptoms to exacerbate for a few days after the challenge with ≤ 5% of asthmatic patients, (Vandenplas *et al.*, 2006). Most asthmatic reactions were resolved with inhalation drugs; only a few reactions have been intravenously treated in our series. In the future the closed-circuit aerosol and vapour exposure chamber system can tailor concentrations of dusts and aerosols, and can further reduce the risk of eliciting > 30% FEV1 falls more than 10%, compared to "the realistic" methodology used in the ICTs analyzed this thesis (Cloutier *et al.*, 1989; Malo *et al.*, 2004).

In the challenge tests studied, IgE mediated organic allergens gave more often positive results. In both nasal provocation tests and inhalation challenge tests studied, the mold challenge reactions were found to have low rate of positive challenge test findings both in nasal and inhalation challenge tests. Our results showed that the majority of the nasal symptoms in moisture-damaged buildings are non-IgE-mediated, as has been previously suggested (Ruoppi et al., 2003). Several indoor air factors, like volatile organic compounds (VOC)s have been suggested to be involved in the aeroirritant effect found in the exposures to fungi (Hope and Simon, 2007), as well as bacterial endotoxins (Pestka, et al. 2008), air CO2 concentration, poor ventilation (Hagerhed-Engman et al., 2009), common indoor contaminants, air humidity, movement and temperature factors (United States Department of Labor, 2008) and psychosocial and personal factors (Ebbehoj et al., 2005). However the mechanisms associated to moisture damages have been also stated to be partly unknown (Bornehag et al., 2001). After these, our and other evaluations of the mould provocations in FIOH, the practice of mold provocations has been changed to be performed to only patients with positive IgE towards the molds.

Only a minor part of respiratory complaints among woodworkers were connected to specific sensitization in our material. In some cases, there may be nasal crusting and dryness, symptoms other than included in the definition of rhinitis, found relevant to the nasal symptoms at work (Hellgren *et al.*, 2003). In our patients with suspicion of occupational rhinitis and positive nasal challenge test to wood dusts, concomitant sensitization to ubiquitous aero-allergens was found in 90%, and concomitant sensitization has also previously been found to be more common among persons with airway diseases from wood dusts compared to the general population (Skovsted *et al.*, 2003). As a result, we suggest that unspecific mucosal hypersensitivity and mechanical and perhaps some hydroscopic irritation of the nasal mucosa are likely causes to some, and to be a major modifier to most of the rhinitis symptoms of these patients in NPT. In sawmills, where fresh wood is handled, also endotoxines, airborne fungi, bacteria and (1.3)-glucan may take part in the airway irritation (Rusca *et al.*, 2008) and symptoms. Yet, the mechanisms behind the irritation caused by wood dusts have been so far only partly resolved.

A recent EAACI position on OR and an earlier paper on provocation tests with allergens give a lot of guidelines to the occupational nasal challenges (Melillo *et al.*, 1997; Moscato *et al.*, 2009). In our study we analyzed the placebo reactions as well as the occupational agent reactions. The results of the placebo reactions have been mainly lacking from the provocation test reports done before. There are some reports describing the effect of several repeated challenge tests enhancing the nasal responsiveness (priming effect) (Ciprandi *et al.*, 1998), but the baseline nasal reactivity of the patients or unspecific nasal hyperreactivity like in relation to nasal comorbidities are seldom documented. Although there are no studies, to our knowledge, in relation to the placebo test criteria, the practice of FIOH has been to repeat the challenge test if the control test was positive and treat all temporary causes of hyperreactivity before the retest. If the hyperreactivity was still present during the control test, the occupational challenge test reaction must be markedly larger than the placebo test reaction, to support or confirm the specific occupational rhinitis. In nasal provocation tests studied, glycerol and phenol containing placebo agents (skin prick test placebo agents used) induced more positive placebo reactions than water-based agents in our results. Therefore, it was suggested that water-based provocation agents only should be used. This is supported by earlier findings (Haahtela and Lahdensuo, 1979).

6.2 Occupational rhinitis and health-related quality of life (III)

The Health-related quality of life (HRQoL) among OR patients had been little studied so far. In the questionnaire study of the thesis it was found that continuing occupational allergen exposure decreased HRQoL among the occupational allergic rhinitis patients and that the HRQoL of patients without occupational allergen exposure were mainly similar than that of the healthy controls. The results highlight the importance of the reduction and cessation of occupational exposure as the main intervention. The result also emphasizes

the determinant role of the occupational allergen to HRQoL. The result is well in line with the study where greenhouse workers' allergic rhinitis-related HRQoL was studied, and the most significant occupational allergen in their work (bell pepper pollen) and common perennial allergies were compared (Groenewoud *et al.*, 2006). In that study, bell pepper pollen allergy had a higher relative impact than the environmental allergies on the Rhinitis Quality of life Questionnaire (RQLQ).

Allergy medication and its use are known to alleviate rhinitis symptoms and improve the HRQoL of rhinitis patients (Tripathi and Patterson, 2001). Antihistamines and nasal steroids are well known as the most effective options for treating AR (Tripathi and Patterson, 2001), although the situation may change. Accordingly, the antihistamines and nasal steroids were the main rhinitis medications used among the patients. Frequency of medication usage was also related to the patients' estimation of their current exposure level. Nevertheless, as the regular use of rhinitis medications was related to lower HRQoL, it seems that they only improve OR, not cure it. Our result strongly points out that in order to improve the quality of life of OR patients, medication only is not a sufficient treatment for OR; reduction or cessation of exposure to the sensitizing agent at the workplace is needed as the main intervention.

The bodily pain score showed the biggest difference in general HRQoL scores. The non-exposed OR patients had statistically less pain compared to the healthy controls. The patients who had not been able to reduce their exposure level since OR diagnosis experienced most bodily pain. Previously in seasonal AR, the HRQoL measured with SF-36 during and outside the pollen season was significantly changed in factors of physical functioning, physical role limitation and bodily pain (Majani *et al.*, 2001). In some other reports, low back pain, and depression or neurosis are significantly linked with allergic pollinosis (Hurwitz and Morgenstern, 1999; Hashimoto *et al.*, 2007). In addition, headache is also 1.5 times more common in persons with AR than among non-allergics (Aamodt *et al.*, 2007). In fact, headache has been found to coexist in 41% of perennial AR patients in a symptom survey and less so in seasonal AR (Scadding *et al.*, 2000). In addition, it may be, that the patients who were not able to reduce their exposure may have more comorbidities, explaining the pain results. In our study the non-exposed OR patients had statistically less pain compared to the healthy controls. This may be explained, on the opposite, by better opportunities to improve the situation, such as change exposure level, lack of allergic symptoms or any comorbidity. The detailed differences in the pain experiences were not specifically questioned in this work, though.

Among occupational latex allergic patients, the complete removal of the exposure has been reported to be statistically related to reduced income, compared to those who had reduced exposure (Vandenplas *et al.*, 2002). Re-education or job change can also have marked professional career consequences: in some reports, it is associated with frequent persistent work disruption. In contrast to this, in our results, the employment rate was similar among the patients and the controls. The occupational rhinitis had caused re-education to 17% of the OR patients though. Out of the re-educated patients, 9% reported increase, 33% decrease, and 58% no changes in livelihood due to OR. In Finland, re-

education due to occupational diseases is discretionarily compensated by mandatory insurance, if the allergen load cannot reasonably be avoided or reduced in the workplace.

The risk of asthma among OR patients is higher than among the general population (Karjalainen *et al.*, 2003). Earlier studies with e.g. RAND-36, state that the more diseases the patient has, the lower their HRQoL is (Aalto *et al.*, 1999). In our study, asthma was related to lower HRQoL in both Rhinasthma and RAND-36, which is in line with previous works.

In the study III, the response rate was quite typical for a questionnaire study. We might have increased the response rate by directly contacting the patients, through calling, or selecting the patient material to include only native Finnish or Swedish persons, or by excluding the eldest group of patients. Anyhow, according to the literature, the nonresponders of respiratory symptom questionnaires may influence the prevalence rates of respiratory symptoms (like with 3-7% underestimation of symptoms, and up to 16% difference in smoking) (Kotaniemi *et al.*, 2001). As in our study the rate of reported asthma was in similar magnitude to the previous reports and smoking did not influence the HRQoL results according to the statistical analyses, we consider that the nonresponders influence to our results was not likely to be significant.

6.3 Occupational wheat allergens

Rhinitis and asthma in bakers and bakery workers is often referred to as baker's rhinitis and baker's asthma. Flours are the main causative agents of these diseases, and wheat is the leading flour used in the western world. Recognition of relevant allergens is important for the development of more specific *in vitro* diagnostics, for the standardization of allergen preparations used and for the possible development of desensitization treatment of baker's respiratory allergy.

Strong cross-reactivity exists between allergens from different cereals, which was also seen in our study. The number of IgE-reactive wheat proteins was found to be high in the patient's sera. Earlier, as many as 40 different antigens have been found and cross-reactivities between other flours and common grass pollen (Blands *et al.*, 1976). Accordingly, in our results some of the patient sera recognized only a few proteins whereas others reacted to several protein spots in immunoblot.

The work was one of the few comparing the clinical importance of various respiratory wheat allergens to the occupationally wheat allergic patients. The occupational wheat allergens are mainly different from the non-occupational diseases oral wheat hypersensitivity and wheat-dependent exercise-induced anaphylaxis, in which gliadins are the major allergen group (Varjonen *et al.*, 2000; Palosuo *et al.*, 2001; Battais *et al.*, 2003). In our study we tested crude commercial gliadin in SPT together with purified water-/ salt

soluble wheat allergens. Fifteen percent of our patients showed some positivity towards gliadin in SPT, but other tested allergens had stronger reactions at the same time. We found this to be in line with the presumption that these proteins are stronger allergens for wheat allergy from oral ingestion rather than respiratory exposure (Palosuo *et al.*, 1999; Palosuo *et al.*, 2001b).

Our study was the first showing that thaumatin-like protein (TLP), to which 45% of our patients recognized as an allergen, is an allergen in baker's respiratory allergy. TLP belongs to plant defence proteins which are important allergens of several fruits such as apples and cherries (Breiteneder, 2004) while in grains they prevent fungal diseases.

The result showed 30% of reactivity to reactivity to purified lipid transfer protein 2G (LTP2G), which had not previously been found as allergen. Another LTP, from water extracts of wheat LTP1 (in allergen nomenclature Tri a14), had previously found as one baker's allergen (Palacin *et al.*, 2007). Our results suggest that several LTP proteins can induce allergy among bakers.

Peroxidase I has previously been found to be a wheat allergen in patients with baker's asthma (Sanchez-Monge *et al.*, 1997; Baur and Posch, 1998; Yamashita *et al.*, 2002). Our results confirmed the earlier findings and concluded that 45% of our patients were sensitized to this allergen.

In an earlier study with a recombinant protein of alpha amylase inhibitor α-AI, 65% of patients with baker's asthma were sensitized to this protein (Weichel *et al.*, 2006). This group of proteins were confirmed to be baker's major allergens also in our study, because 60% of our patients had positive SPT to the alpha-amylase inhibitor proteins.

In addition, our study identified other wheat allergen proteins, whose importance in baker's rhinitis waits for further studies: xylanase inhibitor protein, 1-Cys-peroxiredoxin, gluthatione s-transferase and 27K protein.

6.4 Some unmet needs in OR

This thesis and a recent consensus paper (Moscato *et al.*, 2009) have raised a few unmet needs in the OR field that can be addressed in the future:

- There are no HRQoL studies with all causative agents and longitudinal follow up
- There are no cost-evaluations of the rehabilitation of OR
- The allergen extracts used, methods of measuring non-specific nasal hyperreactivity, nasal inflammation and provocation tests need standardization
- There is a need for an internationally validated questionnaire to identify OR in epidemiologic work and clinical practice
- The diagnosis number of irritation rhinitis is missing in ICD-10
- There is currently no data on nasal NO changes in challenge tests with various chemicals and irritants and versus allergens
- There is a need for prospective immunotherapy studies with occupational allergens
- There is a need for studies assessing parameters influencing the prognosis of OR, and effects of interventions on the development of OA
- There is a need for consensus criteria for grading impairment/ disability resulting from OR

7. Summary and conclusions

I & II

Nearly half of the patients (47%) with upper respiratory related rhinitis symptoms selected to the nasal challenge tests did receive positive reactions suggesting occupational rhinitis in NPTs, but among the patients studied with the inhalation challenge tests positive rhinitis reactions was rarer (13%). The causes of OR were mainly AR.

The specific nasal and inhalation challenge tests were found to be valuable tools when there is uncertainty whether the patient's exposure should be discontinued or the patient should change his or her job. The provocation test mostly does prove or concludes the suspicion of occupational origin of the rhinitis when the allergens are well characterized, and there is no other comorbidity in the nasal mucosa. Both nasal and inhalation challenge test methods were found as safe procedures in our evaluation. Mostly the repetition of the nasal challenge test could clarify the situation, especially if temporary hyperreactivities of the nose are withdrawn. Only markedly stronger provocation test reactions with the occupational agent compared to the control agent reactions can be considered to confirm the suspected OR. That can be interpreted analogous to one SPT criteria where the skin wheal caused by an allergen needs to be markedly larger than the negative control to be considered as a positive reaction.

The nasal provocation test scoring criteria used in FIOH was compared to nasal secretion amount of 0.2g. The scoring criteria used and nasal secretion criteria for nasal provocation tests gave quite similar results. It is in favour that nasal secretion amount is useful, easily used and reliable criteria in the nasal provocation tests.

Provocation tests used were found safe and well-tolerated in our patients. No serious reactions have occurred in these provocation test series. In addition, simultaneous evaluation of both upper and lower airways is cost-effective compared to separate nasal and bronchial challenge tests.

III

Twenty-two percent of the occupational rhinitis (OR) patients were no longer exposed to the occupational agent that had caused their OR on average 10 years after their diagnosis. Seventy-eight percent of the patients had continuing allergen exposure to the agent that had caused their OR. Continuing occupational allergen exposure was related to decreased HRQoL among the patients. The HRQoL of patients without occupational allergen exposure was mainly similar than that of the healthy controls. In addition regular use of rhinitis medications was related to continuing exposure and to lower HRQoL among OR patients. These results strongly point out that in order to improve the quality of life of OR patients, medication only is not a sufficient treatment for OR; reduction or cessation of exposure to the sensitizing agent at the workplace is needed as the main intervention.

The long-term employment rate was similar among the Finnish OR patients and the controls. Seventeen percent of the OR patients were re-educated because of occupational rhinitis. Out of the re-educated patients, 9% reported increase and 33% decrease in livelihood due to OR.

IV

It was confirmed that patients with baker's rhinitis and asthma recognize *in vivo* several different water/salt -soluble proteins from wheat grains. Several recognized allergens, alpha-amylase inhibitors, peroxidase I, thaumatin-like protein, lipid transfer protein 2G and wheat crude gliadin were tested in SPT in baker's respiratory allergy patients. Alpha-amylase inhibitors, lipid transfer protein 2G, thaumatin -like protein, and peroxidase I were found to be relevant allergens in Finnish patients with occupational wheat allergy. Of these allergens, thaumatin-like protein and lipid transfer protein 2G were found as new allergens associated with baker's rhinitis and asthma.

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Appendix 1: Rhinasthma questionnaire used (in Finnish)

Minkä verran seuraavat hengitystieoireisiin liittyvät asiat ovat vaivanneet teitä viimeisten 12 kuukauden aikana? (Ympyröikää sopiva numero)

	1				**5**
	(ei lainkaan)				**(erittäin paljon)**
1. Kurkun selvittäminen	1	2	3	4	5
2. Tarve käyttää lääkkeitä	1	2	3	4	5
3. Tarve käyttää rahaa (lääkkeet, lääkärikäynnit)	1	2	3	4	5
4. Puristava tunne rintakehässä	1	2	3	4	5
5. Hengenahdistus	1	2	3	4	5
6. Silmien kutina	1	2	3	4	5
7. Tarve pitää lääkkeitä mukanaan	1	2	3	4	5
8. Nenän valuminen	1	2	3	4	5
9. Yöheräilyt	1	2	3	4	5
10. Aivastuttaminen	1	2	3	4	5
11. Rajoitukset vapaa-ajan toimiin	1	2	3	4	5
12. Vaikeus nukahtaa	1	2	3	4	5
13. Liikuntasuoritusten heikkeneminen	1	2	3	4	5
14. Tarve nenäliinojen mukana pitämiseen	1	2	3	4	5
15. Rajoitukset päivittäiseen elämään	1	2	3	4	5
16. Keskittymisvaikeudet	1	2	3	4	5
17. Hengästyminen	1	2	3	4	5
18. Tarve välttää tiettyjä ympäristöjä	1	2	3	4	5
19. Nenän tukkoisuus	1	2	3	4	5
20. Hajuaistin heikkeneminen	1	2	3	4	5
21. Huoli ettei olisi lääkettä	1	2	3	4	5
22. Nenän kutina	1	2	3	4	5
24. Tarve käydä lääkärissä	1	2	3	4	5
23. Silmien punoitus	1	2	3	4	5
25. Yskä	1	2	3	4	5
26. Rajoitukset tehdä mitä haluaisi	1	2	3	4	5
27. Lääkkeiden sivuvaikutukset	1	2	3	4	5
28. Silmien arkuus	1	2	3	4	5
29. Hengityksen vinkuminen	1	2	3	4	5
30. Silmien vetistäminen	1	2	3	4	5

Rhinasthma questionnaire used (in Swedish)

I vilken mån har ni besvärats av följande faktorer i samband med symptom i luftvägarna under de sista tolv månaderna? (Inringa det rätta svaret)

	skala: 1...				5
	(inte alls)				(i hög grad)
1. Behov att harkla strupen	1	2	3	4	5
2. Behov att använda mediciner	1	2	3	4	5
3. Behov att använda pengar (mediciner, läkare)	1	2	3	4	5
4. Tryckande känsla i bröstkorgen	1	2	3	4	5
5. Kliande ögon	1	2	3	4	5
6. Andnöd	1	2	3	4	5
7. Behov att ha mediciner till hands	1	2	3	4	5
8. Rinnande näsa	1	2	3	4	5
9. Vaknande om natten	1	2	3	4	5
10. Behov att nysa	1	2	3	4	5
11. Begränsningar som gäller fritidsaktiviteter	1	2	3	4	5
12. Försämrad idrottsprestation	1	2	3	4	5
13. Svårigheter att somna	1	2	3	4	5
14. Behov att ha näsdukar till hands	1	2	3	4	5
15. Begränsningar i det vardagliga livet	1	2	3	4	5
16. Sänkt koncentrationsförmåga	1	2	3	4	5
17. Andfåddhet	1	2	3	4	5
18. Behov att undvika vissa omgivningar	1	2	3	4	5
19. Tilltäppt näsa	1	2	3	4	5
20. Försämrat luktsinne	1	2	3	4	5
21. Rädsla att vara utan medicin	1	2	3	4	5
22. Kliande näsa	1	2	3	4	5
23. Röda ögon	1	2	3	4	5
24. Behov att uppsöka läkare	1	2	3	4	5
25. Hosta	1	2	3	4	5
26. Begränsad möjlighet att göra vad man vill	1	2	3	4	5
27. Biverkningar av medicin	1	2	3	4	5
28. Känsliga ögon	1	2	3	4	5
29. Pipande andetag	1	2	3	4	5
30. Rinnande ögonen	1	2	3	4	5

Appendix 2: RAND-36 1.0 questionnaire (in Finnish)

1. Onko terveytenne yleisesti ottaen ... *(ympyröikää yksi numero)*

1 erinomainen
2 varsin hyvä
3 hyvä
4 tyydyttävä
5 huono

2. Jos vertaatte nykyistä terveydentilaanne vuoden takaiseen, onko terveytenne yleisesti ottaen ... *(ympyröikää yksi numero)*

1 tällä hetkellä paljon parempi kuin vuosi sitten
2 tällä hetkellä jonkin verran parempi kuin vuosi sitten
3 suunnilleen samanlainen
4 tällä hetkellä jonkin verran huonompi kuin vuosi sitten
5 tällä hetkellä paljon huonompi kuin vuosi sitten

Seuraavassa luetellaan erilaisia päivittäisiä toimintoja. Rajoittaako terveydentilannenykyisin suoriutumistanne seuraavista päivittäisistä toiminnoista? Jos rajoittaa, kuinka paljon? *(ympyröikää yksi numero joka riviltä)*

	kyllä, rajoittaa paljon	kyllä, rajoittaa hiukan	ei rajoita lainkaan
3. huomattavia ponnistuksia vaativat toiminnat (esimerkiksi juokseminen, raskaiden tavaroiden nostelu, rasittava urheilu)	1	2	3
4. kohtuullisia ponnistuksia vaativat toiminnat, kuten pöydän siirtäminen, imurointi, keilailu	1	2	3
5. ruokakassien nostaminen tai kantaminen	1	2	3
6. nouseminen portaita useita kerroksia	1	2	3
7. nouseminen portaita yhden kerroksen	1	2	3
8. vartalon taivuttaminen, polvistuminen, kumartuminen	1	2	3
9. noin kahden kilometrin matkan kävely	1	2	3
10. noin puolen kilometrin matkan kävely	1	2	3
11. noin 100 metrin matkan kävely	1	2	3
12. kylpeminen tai pukeutumine	1	2	3

Onko teillä viimeisen 4 viikon aikana ollut RUUMIILLISEN TERVEYDENTILANNE TAKIA alla mainittuja ongelmia työssänne tai muissa tavanomaisissa päivittäisissä tehtävissänne? *(ympyröikää yksi numero joka riviltä)*

	Kyllä	Ei
13. Vähensitte työhön tai muihin tehtäviin käyttämäänne aikaa	1	2
14. Saitte aikaiseksi vähemmän kuin halusitte	1	2
15. Terveydentilanne asetti teille rajoituksia joissakin työ- tai muissa tehtävissä	1	2
16. Töistänne tai tehtävistänne suoriutuminen tuotti vaikeuksia (olette joutunut esim. ponnistelemaan tavallista enemmän)	1	2

Onko teillä viimeisen 4 viikon aikana ollut TUNNE-ELÄMÄÄN LIITTYVIEN vaikeuksien (esim. masentuneisuus tai ahdistuneisuus) takia alla mainittuja ongelmia työssänne tai muissa tavanomaisissa päivittäisissä tehtävissänne?

(ympyröikää yksi numero joka riviltä)	Kyllä	Ei
17. Vähensitte työhön tai muihin tehtäviin käyttämäänne aikaa	1	2
18. Saitte aikaiseksi vähemmän kuin halusitte	1	2
19. Ette suorittanut töitänne tai muita tehtäviänne yhtä huolellisesti kuin tavallisesti	1	2

20. MISSÄ MÄÄRIN ruumiillinen terveydentilanne tai tunne-elämän vaikeudet ovat viimeisen 4 viikon aikana häirinneet tavanomaista (sosiaalista) toimintaanne perheen, ystävien, naapureiden tai muiden ihmisten parissa? *(ympyröikää yksi numero)*

1 ei lainkaan
2 hieman
3 kohtalaisesti
4 melko paljon
5 erittäin paljon

21. Kuinka voimakkaita ruumiillisia kipuja teillä on ollut viimeisen 4 viikon aikana? *(ympyröikää yksi numero)*

1 ei lainkaan
2 hyvin lieviä
3 lieviä
4 kohtalaisia
5 voimakkaita
6 erittäin voimakkaita

22. Kuinka paljon kipu on häirinnyt tavanomaista työtänne (kotona tai kodin ulkopuolella) viimeisen 4 viikon aikana? *(ympyröikää yksi numero)*

1 ei lainkaan
2 hieman
3 kohtalaisesti
4 melko paljon
5 erittäin paljon

Seuraavat kysymykset koskevat sitä, miltä teistä on tuntunut viimeisen 4 viikon aikana. Merkitkää kunkin kysymyksen kohdalla se numero, joka parhaiten kuvaa tuntemuksianne. *(ympyröikää yksi numero joka riviltä).*

Kuinka suuren osan ajasta olette viimeisen 4 viikon aikana ...

	koko ajan	suurimman osan aikaa	huomattavan osan aikaa	jonkin aikaa	vähän aikaa	en lainkaan
23. tuntenut olevanne täynnä elinvoimaa	1	2	3	4	5	6
24. ollut hyvin hermostunut	1	2	3	4	5	6
25. tuntenut mielialanne niin matalaksi, ettei mikään ole voinut teitä piristää	1	2	3	4	5	6
26. tuntenut itsenne tyyneksi ja rauhalliseksi .	1	2	3	4	5	6
27. ollut täynnä tarmoa	1	2	3	4	5	6
28. tuntenut itsenne alakuloiseksi ja apeaksi....	1	2	3	4	5	6
29. tuntenut itsenne “loppuunkuluneeksi”.....	1	2	3	4	5	6
30. ollut onnellinen	1	2	3	4	5	6
31. tuntenut itsenne väsyneeksi.....	1	2	3	4	5	6

32. Kuinka suuren osan ajasta ruumiillinen terveydentilanne tai tunne-elämän vaikeudet ovat viimeisen 4 viikon aikana häirinneet tavanomaista sosiaalista toimintaanne (ystävien, sukulaisten, muiden ihmisten tapaaminen)? *(ympyröikää yksi numero)*

1 koko ajan
2 suurimman osan aikaa
3 jonkin aikaa
4 vähän aikaa
5 ei lainkaan

Kuinka hyvin seuraavat väittämät pitävät paikkansa teidän kohdallanne?
(ympyröikää yksi numero joka riviltä)

	pitää ehdottomasti paikkansa	pitää enimmäkseen paikkansa	en osaa sanoa	enimmäkseen ei pidä paikkansa	ehdottomasti ei pidä paikkansa
33. Minusta tuntuu, että sairastun jonkin verran helpommin kuin muut ihmiset	1	2	3	4	5
34. Olen vähintään yhtä terve kuin kaikki muutkin tuntemani ihmiset	1	2	3	4	5
35. Uskon, että terveyteni tulee heikkenemään	1	2	3	4	5
36. Terveyteni on erinomainen	1	2	3	4	5